THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

	PAGE
THE EFFECT OF LIGHT AND HEAT ON THE EXCRETION OF PARENTERALLY INTRODUCED PHOSPHATES. *Otakar Barkus and Frank C. Balderrey*	425
PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE. V. THE PHYSICAL AND CHEMICAL NATURE OF THE LUCIFERINE OF CYPRIDINA HILGENDORFII. *Sakyo Kanda*	435
FACTORS CONCERNED IN BLOOD VOLUME REGULATION. *Alfred Chanutin, Arthur H. Smith and Lafayette B. Mendel*	444
SUPRARENAL ENLARGEMENT UNDER HEAVY DOSAGE WITH INSULIN. *Oscar Riddle, Hannah Elizabeth Honeywell and Walter S. Fisher*	461
CHEMICAL CHANGES IN THE BLOOD DURING FASTING AND SUBSEQUENT REFEEDING. EXPERIMENTS ON DOGS. I. *Sergius Morgulis and A. C. Edwards*	477
STUDIES OF THE BRAIN STEM. VIII. DIURESIS AND ANHYDREMIA FOLLOWING DESTRUCTION OF THE THALAMUS. *Fred T. Rogers*	490
STUDIES OF THE BRAIN STEM. IX. ON THE RELATION OF CEREBRAL PUNCTURE HYPERTHERMIA TO AN ASSOCIATED ANHYDREMIA. *Fred T. Rogers*	507
THE INFLUENCE OF PREGNANCY AND LACTATION ON THE WEIGHT OF ADRENAL GLANDS IN THE ALBINO RAT. *John C. Donaldson*	517
ON GLOMERULAR FILTRATION. *H. L. White*	523
ABSORPTION OF INSULIN BY RECTUM. *S. Peskind, J. M. Rogoff and G. N. Stewart*	530
THE EFFECTS OF INTRAVENOUS INJECTIONS OF GLUCOSE AT A CONSTANT RATE ON BLOOD SUGAR AND HEMOGLOBIN CONCENTRATIONS. *E. C. Albritton*	542
STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS. XIX. A HITHERTO UNKNOWN FUNCTION OF THE THYMUS. *Oscar Riddle*	557
CARDIO-VASCULAR REACTIONS TO HEMORRHAGE AND TRANSFUSION IN MAN. *J. A. E. Eyster and W. S. Middleton*	581
STUDY OF THE MECHANISM OF CHANGE IN RESISTANCE OF ERYTHROCYTES TO HYPOTONIC SALT SOLUTION. III. A STUDY OF THE CAUSE OF EFFECTS PRODUCED BY CATIONS ON THE RESISTANCE OF RED CORPUSCLES PREVIOUSLY DESCRIBED. *Winifred Ashby*	585
A STUDY OF THE MECHANISM OF CHANGE IN RESISTANCE OF ERTHROCYTES TO HYPOTONIC SALT SOLUTION. IV. ON A RADICAL DIFFERENCE IN THE EFFECT OF UNIVALENT CATIONS WITH REFERENCE TO RESISTANCE OF THE CORPUSCLES OF CERTAIN ANIMALS, WHICH IS ASSOCIATED WITH DIFFERENCES IN THE NORMAL METAL CONTENT OF THE RESPECTIVE CORPUSCLES; AND THE SIMILARITY OF THE EFFECT OF THE BIVALENT CATIONS. *Winifred Ashby*	611
STUDIES ON CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS. XII. CHANGES IN METABOLISM FOLLOWING ADRENAL STIMULATION. *Monroe A. McIver and Elizabeth M. Bright*	622
THE COLLOID CHEMISTRY OF PROTOPLASM. III. THE VISCOSITY OF PROTOPLASM AT VARIOUS TEMPERATURES. *L. V. Heilbrunn*	645
INDEX	649

VOL. LXVIII—No. 3

Issued May 1, 1924

BALTIMORE, U. S. A.

1924

ered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917 Authorized on July 5, 1918

Made in United States of America

MERCURY MANOMETER WITH THREE-WAY COCK

THIS manometer is constructed of carefully selected calibrated, annealed glass tubing, which is mounted on a hard rubber block. The horse-hair and weight of the actual manometer are replaced by a slotted brass tube which serves as a guide for the writing needle, assuring continuous approximation of writing point to paper. The writing point is brought into contact with the paper by turning a screw which rotates the brass guide tube. The scale is not fixed, as in other types of manometer, but can be adjusted at "blood pressure zero" for each experiment. The three-way cock serves to put into communication the anti-clot reservoir, the manometer and the blood vessel.

PRICE $23.00

Catalogue on Request

JOSEPH BECKER

437 WEST 59TH STREET **NEW YORK**

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 68 MAY 1, 1924 No. 3

THE EFFECT OF LIGHT AND HEAT ON THE EXCRETION OF PARENTERALLY INTRODUCED PHOSPHATES

OTAKAR BARKUS AND FRANK C. BALDERREY

From the Department of Research, J. N. Adam Memorial Hospital, Perrysburg, N. Y.

Received for publication January 25, 1924

In a previous communication we demonstrated the disappearance from the blood stream of parenterally introduced simple salts under the influence of sun and artificial light. The question was left open whether this phenomenon is due to an increased renal activity or to increased permeability and diffusion between blood stream and tissue spaces.

That renal activity is influenced by various stimuli such as heat, cold and radiant energy was shown by earlier investigators. Lambert (1) reports increased urinary secretion after prolonged refrigeration; Delezenne (2) found a decrease in urinary secretion due to short chills; the increase was explained by Lambert by the experimentally ascertained vasoconstriction of the kidney and increased arterial blood pressure, factors balancing each other; however, after prolonged refrigeration, increased arterial blood pressure predominated, causing increased secretory activity; the stage of vasoconstriction due to short chills explains the observation of Delezenne on decreased renal function under this condition. Lenkei (3) observed an increase in volume, total solids and urea when exposing an individual to a total air bath at a temperature of 10° to 13.2°; the same findings were made at a temperature between 15.8° and 20.0°; when exposing the same man to a total air bath at 20° to 28.0° without exposure to direct sunlight a decrease in volume, urea and total solids was observed. Denoyes, Martre and Rouvière (4) studied the influence of high frequency currents on urinary secretion; they report an increase in volume, urea, uric acid total nitrogen, phosphate, sulfate and chloride during the period of treatment; this effect continued for three days after treatment was stopped. McQuarrie and Whipple (5) studied the influence of Röntgen rays on renal function in the dog; they summarize their results as follows:

With x-ray exposures of the abdomen and shielding of the kidneys fatal intoxication may be produced without the slightest disturbance of kidney function as measured by the ability of the kidney to eliminate phenolphthalein and urea. Larger doses of x-rays given directly over the kidney may cause a slight but distinct lowering of renal function which lasts for a period of a few days. We have been unable to recognize any corresponding histological change.

The effects of exposure to x-ray, heat and high frequency currents follow on the whole the Arndt-Schulz law; not so the effects of exposure to visible light where several components are to be considered; first, the effect of irradiation with light of the short wave length region causing vasodilatation as proven by Bernard (6), Finsen (7), Hasselbalch and Jacobäus (8) and collateral hyperemia of the secretory organs as shown by Gassul (9), Levy (10), and experiments performed in this laboratory as yet unpublished; second, the effect on arterial blood pressure of light of the same region studied by Austgen (11), Bach (12), Kimmerle (13), Günther (14), A. Meyer (15), Löwenhardt (16) and Peemöller (17), all of whom observed a definite lowering; Günther (14) points out that the lowering of blood pressure as effected by irradiation with artificial light (1500 candles) follows the tonus curves of vasodilatation, i.e., a short negative phase followed by a long positive phase; third, the effects of heat and irradiation with light of long wave-length which causes increase in arterial blood pressure and in blood viscosity. Arrak (18) immersed normal and nephropathic men in water baths of different temperatures; up to 30° a decrease in blood pressure was observed; from 30° to 50° a definite increase occurred in both groups. Bering (19) found after prolonged irradiation of mice with the Alpine lamp an increase in the hemoglobin content and erythrocytes. Riedel (20) reports a high hemoglobin index after solar irradiation but only a very slight increase in the color index after exposure to the mercury arc. Burchardi (21) reports the same observation after exposure to intensive carbon arc light. Young, Breinl, Harris and Osborne (22) found in a long series of experiments performed on man in an ironclad hot room filled with steam of various temperatures that increase in blood pressure and pulse frequency goes parallel with increase in rectal temperature. During prolonged exposure of an animal to visible light there will probably occur, first, vasodilatation followed by diffusion of tissue fluid into the blood stream causing blood dilution and increased blood volume; and hyperemia of the skin and collateral hyperemia of the secretory organs, then increased catabolism and water production, resulting in blood dilution which may, however, at this point be counteracted by general overheating, causing vasoconstriction, diffusion of water and electrolytes into the tissue spaces and blood thickening.

A series of experiments was, therefore, undertaken to ascertain whether the increased blood volume and increased blood flow mentioned above stimulate renal activity.

Full-grown female sheep were infused with a total of 2.00 gP, the technique being the same as described in the first paper (23). The animal was catheterized and the catheter kept in place by means of adhesive tape; the urine was collected in small flasks attached to the table and the collecting flask changed at intervals of 30 minutes; complete drainage was effected by pressure on the abdominal wall and moving of the catheter. The volume of the specimen was recorded and the phosphates determined by Jones' method. Ten days later the same animal was used for a "light" experiment; the sheep was shaved as described in the first paper and exposed to the light of 4 open carbon arc lamps (110 volts, 10 amp.) such as are used for street illumination, at 50 cm. skin distance. Irradiation began 60 minutes before infusion was made and lasted 360 to 480 minutes. The results of this series are given in table 1. The columns 30, 60, 120, 180, 240, 300, 360 and 420 designate in minutes the period elapsed after infusion. Under "volume of urine" and "phosphorus output" the relative output of each is registered, the total of urine and phosphorus respectively being set at 100 per cent (e.g., sheep, blue 3; D; total volume of urine excreted was 330 cc. = 100 per cent; total phosphorus excreted was 0.3864 g = 100 per cent: the relative amounts of the individual specimen are calculated on this basis). The last column "total" gives the actual volume of urine and the percentage of phosphorus excreted (e.g., 27.3 per cent of the P infused was excreted within 420 minutes). The letters D and L stand for "dark" and "light" experiment and relate, of course, to the horizontal rows. The number under "rectal temp." gives the temperature as observed at the time when the collecting flask was changed (e.g., R. temp. 40.0° under 120 means temperature 120 minutes after infusion or 180 minutes after exposure). This method of recording was chosen because it gives a good picture of the quality and quantity of diuresis.

The experimental results as far as water diuresis is concerned are not very conclusive. The animals were infused with equal doses of phosphorus and water; conditions during "D" and "L" experiments were equal; considering these facts it is difficult to understand the discrepancy in water diuresis in blue 3 and brown 2 when compared with brown 1 and red 1. The total amount of water excreted during "L" experiments is slightly increased in the former, but only half or one-third of the "D" output in the latter. This might be explained by assuming an increased catabolism or that the water produced during an active catabolic or diuretic period is in one animal excreted to a larger extent through the kidney, in the other through the lungs. That increased catabolism plays a great rôle can be seen when the amounts of water excreted during the last period (300 to 420 minutes) i.e., after a total irradiation of 360 minutes or 480 minutes respectively, are compared; the numbers under "L" are

always larger than those under "D" independent of the difference in total water output.

The quality of water diuresis is not very much changed in "L" experiments; not so the quality of the salt diuresis; the latter always reaches its peak within the first period during the "L" experiments but only within the second period during the "D" experiments; the quality of salt diuresis differs inasmuch as the total output of phosphorus is always larger during "L" than during "D." This tends to show that with qualitatively

TABLE 1

		30	60	120	180	240	300	360	420	TOTAL
Sheep, blue 3:										
Volume of	D		22.2	10.0	11.5	14.5	27.5		14.3	330 cc.
Urine	L		20.0	19.7	11.7	15.8	12.5		20.3	387 cc.
Output of	D	33.8	*52.0*	2.8	3.9				7.5	21.0 per cent
Phosphorus	L	*44.9*	21.0	27.1	4.2				2.8	27.3 per cent
Rectal temp	L	Temperature not taken								
Sheep, brown 1:										
Volume of	D		20.0	14.4	41.1	14.2	5.4	4.8		756 cc.
Urine	L		18.3	18.4	9.6	14.9	14.3	14.5		261 cc.
Output of	D	29.3	*49.5*	13.2	5.4			2.6		19.3 per cent
Phosphorus	L	*61.2*	18.8	9.9	2.7			7.4		20.0 per cent
Rectal temp	L	39.8		40.0		40.2		40.2		n = 39.2°
Sheep, brown 2:										
Volume of	D		54.2	16.5	12.6	7.3	9.4			382 cc.
Urine	L		45.5	17.9	11.8	12.1	12.7			448 cc.
Output of	D	33.4	*41.9*	17.3	5.6		1.8			21.1 per cent
Phosphorus	L	*52.6*	27.7	15.8	2.1		1.8			25.3 per cent
Rectal temp	L	39.8	40.2	40.6	40.8	40.0	41.2			n = 39.6°
Sheep, red 1:										
Volume of	D		30.8	13.9	13.8	17.1	11.9		12.5	757 cc.
Urine	L		30.0	15.1	11.0	11.7	11.0		21.2	410 cc.
Output of	D	31.5	*35.0*	23.7	7.1				2.7	19.5 per cent
Phosphorus	L	*40.8*	24.3	23.9	7.4				3.6	24.1 per cent
Rectal temp	L	40.0	40.1	40.2	40.3	40.1	39.8			n = 39.5°

unchanged water diuresis an increased output of phosphorus takes place during the "L" experiments.

A series of experiments was undertaken to study the influence of irradiation with the carbon arc on the concentration ratio and the ratio between volume and chloride concentration. The technique in these experiments was the same as in previous work, but the sheep were infused with only 0.4971 gP as Na_2HPO_4 in a total volume of 50 cc. distilled water adjusted to pH 7.3. The phosphorus in urine and plasma was determined in

triplicate and duplicate respectively by Bell and Doisy's method; the chlorides in urine by Folin's variation of the Volhardt titration. The urine contained at times, usually in the first and second period, a sediment of double phosphates; this was separated by centrifugalization and the amount of phosphorus in the sediment determined and added to the phosphorus of the supernatant fluid. The volume was measured with certified cylinders before centrifugalization. The protocol tables contain under "interval" the time elapsed after infusion; the remarks: "volume or urine passed" "conc. of P in urine, of Cl in urine" "total P excreted" "Cl excreted" and "conc. of P in plasma" relate to specimen taken at the end of the respective period; concentration ratios are calculated from the urine and plasma concentration of the same period; rectal temperature was taken at the end of each period. For each light experiment a dark control experiment was performed on the same animal; the results of these control experiments are recorded in brackets in the corresponding spaces. Irradiation varied from a 260 minute exposure at a low room temperature (16° to 18°) to a prolonged exposure of 400 minutes at a room temperature of 30° to 33°. The animals were evidently overheated during the long exposure at 30° to 33°; respiratory function and heart action were very much accelerated, though regular during experiments 3 and 4.

It will be seen that a higher concentration ratio resulted in all "L" experiments irrespective of body or room temperature. The total phosphorus output is with the exception of experiment 2 greater during exposure. Water diuresis is qualitatively changed and quantitatively diminished during exposure in experiment 1, changed and increased in experiment 2; definitely decreased—as could be expected—in the overheated animals. Chlorine diuresis (i.e., total, periodic output of Cl) goes parallel with the water diuresis, the concentration of various specimens depending on the urinary phosphorus concentration, a high phosphorus output being equalized by a small chlorine excretion; the chlorine excretion tends, however, always to return to a higher level after such periods.

To summarize our findings on renal function: exposure to the carbon arc causes a high concentration ratio relative to intravenously introduced hypertonic phosphate solutions; this phenomenon is independent of body temperature and water and chlorine diuresis. The relation between chlorine and water diuresis remains, with the exception of the highly concentrated urines of experiment 3, unchanged. It is interesting to compare the total phosphorus excretion with the corresponding plasma phosphorus concentration. The rapid fall in plasma phosphorus concentration is not followed by an increased total urinary phosphorus output in the first periods of experiments 1 and 2. In the overheated animals in experiments 3 and 4 disappearance of phosphorus from the blood stream is rather slow,

TABLE 2

Experiment 1

January 14, 1924. Sheep, brown 3; exposed to the carbon arc for 260 minutes and infused as on January 8.

NUMBER	INTERVAL	VOLUME OF URINE PASSED	CONCENTRATION OF P IN URINE	CONCENTRATION OF Cl IN URINE	TOTAL P EXCRETED	TOTAL Cl EXCRETED	CONCENTRATION OF P IN PLASMA	CONCENTRATION RATIO	RECTAL TEMPERATURE
	minutes	*cc.*	*mg./100*	*mg./1000*	*mg.*	*mg.*	*mg./100*		*degrees*
N.	0						(5.9) 4.7		38.9
1	15						(10.5) 10.8		
2	30	(70) 50	(54.1) 71.0	(655) 405	(37.9) 36.0	(45.9) 20.25	(10.4) 8.4	(5.2) 8.5	39.1
3	45						(10.7) 8.1		
4	60	(80) 28	(14.6) 17.9	(225) 355	(11.68) 16.63	(18.0) 9.9	(8.8) 8.3	(1.7) 2.2	39.3
5	90	(61) 28	(4.2) 5.2	(150) 300	(2.56) 1.46	(9.2) 8.4	(7.7) 6.4	(0.6) 0.8	
6	120	(48) 40	(6.8) 4.8	(255) 295	(3.25) 1.92	(12.2) 11.8	(7.2) 5.5	(0.9) 0.8	
7	150						(6.4) 5.2		
8	180	(75) 32	(6.6) 17.6	(355) 455	(4.95) 5.63	(26.6) 14.56	(6.7) 5.1	(1.0) 3.4	39.2
9	240	(94) 34	(5.2) 8.9	(205) 450	(4.89) 3.03	(19.3) 15.3	(5.5) 4.6	(0.9) 1.9	39.1

Room temperature: 16–18°.

TABLE 3

Experiment 2

January 10, 1924. Sheep, brown 1; exposed to the carbon arc for 280 minutes and infused as on January 4.

NUMBER	INTERVAL	VOLUME OF URINE PASSED	CONCENTRATION OF P IN URINE	CONCENTRATION OF Cl IN URINE	TOTAL P EXCRETED	TOTAL Cl EXCRETED	CONCENTRATION OF P IN PLASMA	CONCENTRATION RATIO	RECTAL TEMPERATURE
	minutes	*cc.*	*mg./100*	*mg./1000*	*mg.*	*mg.*	*mg./100*		*degrees*
N	0						(4.0) 4.6		39.6
1	15						(10.7) 9.0		
2	30	(60) 50	(65.0) 67.0	(480) 225	(39.0) 21.5	(28.8) 11.3	(10.1) 8.4	(6.4) 8.0	39.8
3	45						(8.9) 7.5		
4*	60	(48) 96	(33.5) 4.0	(216) 115	(16.1) 3.84	(10.4) 11.1	(8.3) 7.2	(4.0) 0.6	
5	90	(36) 58	(5.4) 4.1	(434) 155	(1.94) 2.38	(14.6) 9.0	(8.0) 6.6	(0.7) 0.6	
6	120	(30) 44	(6.7) 4.0	(480) 110	(1.91) 1.76	(14.4) 4.8	(7.5) 6.1	(0.9) 0.7	
7	150						(6.8) 5.7		
8	180	(55) 121	(7.2) 4.0	(434) 255	(3.96) 4.84	(23.9) 30.7	(6.1) 5.2	(1.2) 0.8	40.0
9	240	(50) 112	(8.5) 4.5	(580) 225	(4.25) 5.05	(29.0) 25.2	(5.7) 5.4	(1.5) 0.8	40.1

Room temperature: 16°.

TABLE 4

Experiment 3

January 15, 1924. Sheep, blue 3; exposed to the carbon arc for 90 minutes previous to experiment and for a total of 330 minutes, infused as on January 9.

NUMBER	INTERVAL	VOLUME OF URINE PASSED	CONCENTRATION OF P IN URINE	CONCENTRATION OF Cl IN URINE	TOTAL P EXCRETED	TOTAL Cl EXCRETED	CONCENTRATION OF P IN PLASMA	CONCENTRATION RATIO	RECTAL TEMPERATURE
	minutes	*cc.*	*mg./100*	*mg./1000*	*mg.*	*mg.*	*mg./100*		*degrees*
N.	0						(4.3) 4.0		38.4
1	15						(10.1) 10.7		39.8
2	30	(32) 16	(15.2) 205.0	(625) 1455	(4.86) 32.8	(20.0) 23.3	(9.0) 9.8	(1.7) 21.0	40.6
3	45						(8.5) 8.8		
4	60	(28) 27	(10.3) 15.3	(505) 1115	(2.88) 4.13	(14.2) 30.1	(7.8) 8.5	(1.3) 1.8	
5	90	(20) 23	(7.8) 10.4	(620) 1010	(1.56) 2.4	(12.4) 23.2	(7.1) 8.0	(1.1) 1.3	40.4
6	120	(17) 12	(7.7) 10.0	(620) 1120	(1.31) 1.2	(10.6) 13.4	(5.9) 7.4	(1.3) 1.4	
7	150						(5.5) 6.4		40.0
8	180	(42) 21	(9.7) 9.6	(805) 1015	(4.07) 2.0	(33.8) 21.3	(5.5) 5.7	(1.9) 1.7	
9	240	(36) 37	(12.9) 9.2	(755) 1250	(4.64) 3.4	(27.2) 46.3	(4.9) 4.8	(2.6) 1.9	40.1

Room temperature: 25–30°.

TABLE 5

Experiment 4

January 11, 1924. Sheep, red 1; exposed to carbon arc for 160 minutes previous to experiment and kept under arc for a total of 400 minutes; infused as on January 7.

NUMBER	INTERVAL	VOLUME OF URINE PASSED	CONCENTRATION OF P IN URINE	CONCENTRATION OF Cl IN URINE	TOTAL P EXCRETED	TOTAL Cl EXCRETED	CONCENTRATION OF P IN PLASMA	CONCENTRATION RATIO	RECTAL TEMPERATURE
	minuets	*cc.*	*mg./100*	*mg./1000*	*mg.*	*mg.*	*mg./100*		*degrees*
N.	0						(4.6) 3.8		38.6
1	15						11.2		
2	30	(56) 45	(38.1) 79.1	(655) 365	(21.4) 35.6	(36.6) 16.4	10.0		39.3
3	45						(10.5) 9.7	(3.6) 8.2	
4	60	(32) 25	(8.0) 14.2	(565) 255	(2.56) 3.55	(18.1) 6.4	8.9		40.6
5	90	(27) 21	(5.6) 14.3	(450) 365	(1.51) 3.0	(12.2) 7.7	(6.8) 7.7	(0.8) 1.9	
6	120	(27) 12	(6.2) 13.6	(555) 515	(1.67) 2.44	(15.0) 6.2	7.6		41.6
7	150						5.9		
8	175	(43) 13	(9.5) 7.7	(455) 810	(4.09) 1.0	(19.6) 10.5	(5.7) 5.5	(1.7) 1.4	
9	240	(50) 18	(10.7) 4.4	(715) 865	(5.35) 0.89	(35.8) 15.6	(4.7) 4.3	(2.3) 1.0	41.6

Room temperature: 30–33°.

and the total urinary phosphorus output is large in experiment 3 (period I) but small in experiment 4 (same period). In both experiments thickening of the blood as evidenced by a high hemoglobin index (gasometrically determined) occurred; the decrease in plasma phosphorus concentration in experiments 1 and 2 is, therefore, probably due to dilution resulting from vasodilatation and diffusion of water from the tissues rather than to increased elimination.

BIBLIOGRAPHY

(1) LAMBERT: Arch. d. phys. norm. et path., 1897, 128.
(2) DELEZENNE: Compt. rend. Soc. de biol., 1894, vi, 46.
(3) LENKEI: Berich. d. V. Kongr. f. Thalassotherapie.
(4) DENOYES, MARTRE AND ROUVIÈRE: Compt. rend. Acad. des Sci., 1901, cxxxiii, 64.
(5) MCQUARRIE AND WHIPPLE: Journ. Exper. Med., 1922, xxxv, 2.
(6) BERNARD: Sonnelichtbehandlung i. d. Chir., 1917, Stuttgart.
(7) FINSEN: Finsen's Mitt., 1900, i, 8.
(8) HASSELBALCH AND JACOBÄUS: Berl. klin. Wochenschr., 1907, 39.
(9) GASSUL: Strahlentherap., 1919, ix, 223.
(10) LEVY: Strahlentherap., 1916, vii; 1919, ix.
(11) AUSTGEN: Beitr. z. kl. d. Tuberkulose, xlii, i, 18.
(12) BACH: Deutsch. med. Wochenschr., 1911, 9.
(13) KIMMERLE: Strahlentherap., 1921, xiii, 290.
(14) GÜNTHER: Med. Klinik, 1923, 9.
(15) MEYER: Münch. med. Wochenschr., 1922, 23.
(16) LÖWENHARDT: Dissertation, Greifswald, 1918.
(17) PEEMÖLLER: Klin. Wochenschr., 1923, ü, 21.
(18) ARRAK: Zeitschr. f. klin. Med., 1923, xcvi, 416.
(19) BERING: Med. naturw. Arch., 1907, i, 131.
(20) RIEDEL: Strahlentherap., 1921, xii, 361.
(21) BURCHARDI: Strahlentherap., 1921, xii, 808.
(22) YOUNG, BREINL, HARRIS AND OSBORNE: Proc. Roy. Soc., ser. B., xci, 1920, 636.
(23) BARKUS AND BALDERREY: This Journal, 1924, lxvii, 608.

PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE

V. The Physical and Chemical Nature of the Luciferine of Cypridina Hilgendorfii

SAKYO KANDA

From the Marine Biological Laboratory, Kyushu Imperial University, Tsuyazaki (Fukuoka), Japan

Received for publication January 29, 1924

Introductory. As the writer already stated in his first paper of this series, the solutions of the luciferine and luciferase of Cypridina hilgendorfii extracted with hot and cold water are certainly positive to some protein color tests (4, p. 557). It is impossible, however, to identify either luciferine or luciferase with a protein in solution, because the solution is impure. It is therefore essential to look for a way in which the pure Cypridina luciferine can be isolated.

Unfortunately, the writer cannot definitely state that he has isolated the pure luciferine. Nevertheless it is certain that the way of isolating the pure luciferine is approaching step by step. These points will be made clear at due places. The writer is still continuing his investigation but he will report his results obtained as far as his present experiments are concerned.

Isolation with methyl alcohol. The living animals are thoroughly dried in direct sunlight. They die while drying. The dried animals are kept in desiccators over $CaCl_2$. The "shells" of the animals are crushed and are carefully sieved off. The body parts of the animals extracted thoroughly with several changes of ether in the course of a few days and then with petroleum-ether and benzene are kept in ether as raw material for the isolation of the luciferine and further experiments.

This raw material is ground to a powder in a mortar in order to facilitate the extraction of the luciferine. This powdered material is extracted with absolute methyl alcohol for six hours and is filtered off twice with a double filter paper. The filtrate is perfectly clear and is reddish yellow in color. It contains the luciferine, as it produces a brilliant light with the Cypridina luciferase.

This filtrate is evaporated to dryness on the water bath and the residue may be kept intact over ninety days in the desiccator over $CaCl_2$. The residue incompletely dissolves in redistilled water saturated with H_2. The luciferine remains intact in this solution for a few hours and produces

a bright light if the luciferase is added. The luciferine in this solution is completely precipitated by phosphotungstic acid and on saturation with powdered $(NH_4)_2SO_4$, but not by $K_4Fe(CN)_4$, picric acid, heavy metal salts, acids or alkalis. This solution in question gives Millon and ninhydrin reactions, but no biuret or tryptophane. Special care should be taken not to heat the mixture of the solution and Millon's reagent to the point of boiling. If heated to the point of boiling, the color never appears.

That the solution contains a protein or protein derivatives is therefore certain. The question then arises whether the protein color reactions of the solution are due to the presence of the luciferine or to something else; in other words, whether the luciferine is the protein which gives the Millon and ninhydrin reactions or not. So far as the results of the experiments are hitherto mentioned, there are no definite means of identification of the luciferine as the protein of the solution. The results are summarized in table 1.

Isolation in combination. The luciferine is also extracted by absolute ethyl alcohol. The results obtained from the experiments on this extract are quite similar to the results obtained from those on absolute methyl alcohol extract. It is true, however, that absolute methyl alcohol can extract the luciferine very much faster than absolute ethyl alcohol can do, though the former extract contains more substance or substances besides the luciferine than the latter does. The writer, therefore, made use of the peculiarities of these solvents combined for the isolation of the luciferine.

That is to say, absolute methyl alcohol extract is filtered twice with a double filter paper and the perfectly clear filtrate, say 100 cc., evaporated down to a "chyme" of 2 or 3 cc. on the water bath. Absolute ethyl alcohol in excess, say 150 cc., added to this chyme gives a voluminous precipitate. It settles on the bottom of the beaker for five or ten minutes. The supernatant fluid is carefully filtered off with a double filter paper and is kept for further experiments. This precipitate is thoroughly washed with absolute ethyl alcohol on the filter and is incompletely dissolved in redistilled water saturated with H_2. This solution contains some protein or protein derivatives, as it gives Millon and ninhydrin reactions, but no luciferine.

On the other hand, the perfectly clear filtrate of the supernatant fluid is evaporated almost to dryness on the water bath. This residue dissolves incompletely in redistilled water saturated with H_2. This solution contains the luciferine, as it produces a brilliant light if the luciferase is added. It also gives Millon and ninhydrin reactions. The luciferine in this solution completely precipitates on saturation with powdered $(NH_4)_2SO_4$. That is to say, the perfectly clear filtrate gives no light if the luciferase is added. But it still gives Millon and ninhydrin reactions. The substance of this filtrate which gives Millon and ninhydrin reactions may be a peptone even though it gives no biuret test.

TABLE 1

Nature of the Cypridina luciferine

EXPERIMENTS	LUCIFERINE	
	The extract of absolute methyl alcohol evaporated to dryness and dissolved in H_2O saturated with H_2. This solution indicated by (A)	The (B) of table 2 dissolved in H_2O saturated with H_2 or in absolute methyl or ethyl alcohol
Biuret reaction	Negative	Negative
Tryptophane reaction	Negative	Negative
Xanthoproteic reaction	Negative	Negative
Millon reaction	Positive	Negative
Ninhydrin reaction	Positive	Negative
Molisch reaction	Positive	Positive ?
Saturation with NaCl	Not precipitated	Not precipitated
Saturation with NaCl and acetic acid	Not precipitated	Not precipitated
Saturation with NaCl and acetic acid or HCl	Not precipitated	Not precipitated
Half-saturation with $MgSO_4$	Not precipitated	Not precipitated
Saturation with $MgSO_4$	Not precipitated	Not precipitated
Saturation with $MgSO_4$ and acetic acid	Not precipitated	Not precipitated
Saturation with $MgSO_4$ and acetic acid or HCl	Not precipitated	Not precipitated
Half-saturation with $(NH_4)_2SO_4$	Not precipitated	Not precipitated
Saturation with $(NH_4)_2SO_4$	Completely precipitated	Completely precipitated
Phosphomolybdic acid		Not precipitated
Phosphomolybdic acid and HCl		Not precipitated ?
Phosphotungstic acid	Completely precipitated	Not precipitated ?
Phosphotungstic acid and HCl		Not precipitated ?
Tannic acid	(?)	Not precipitated
Tannic acid and HCl	(?)	Not precipitated ?
Picric acid	Not precipitated	
Picric acid and HCl	Not precipitated	
$K_4Fe(CN)_6$	Not precipitated	Not precipitated
$K_4Fe(CN)_6$ and acetic acid or HCl	Not precipitated	Not precipitated
$HgCl_2$	Not precipitated	Not precipitated
$HgCl_2$ and NaOH	Not precipitated	Not precipitated
$HgCl_2$ and acetic acid	Not precipitated	Not precipitated
Pb acetate	Not precipitated	Not precipitated
Pb acetate and NaOH	(?)	(?)
Pb acetate and acetic acid	Not precipitated	Not precipitated
$CuSO_4$	Not precipitated	Not precipitated

TABLE 1—*Concluded*

EXPERIMENTS	LUCIFERINE	
	The extract of absolute methyl alcohol evaporated to dryness and dissolved in H_2O saturated with H_2. This solution indicated by (A)	The (B) of table 2 dissolved in H_2O saturated with H_2 or in absolute methyl or ethyl alcohol
$ZnSO_4$	Not precipitated	Not precipitated
$FeCl_3$	Not precipitated	Not precipitated
$AgNO_3$	(?)	(?)
HCl	Not precipitated	Not precipitated
H_2SO_4	Not precipitated	Not precipitated
HNO_3	Not precipitated	Not precipitated
H_2CO_3	Not precipitated	Not precipitated
Acetic acid	Not precipitated	Not precipitated
KOH	Not precipitated	Not precipitated
NaOH	Not precipitated	Not precipitated
NH_4OH	Not precipitated	Not precipitated

The blank places indicate that no experiments were made.

The precipitate caused on saturation with powdered $(NH_4)_2SO_4$ is thoroughly washed with the saturated solution of $(NH_4)_2SO_4$ on the filter until Millon and ninhydrin reactions are not detected any more in the washed out alcohol solution. The precipitate unremoved from the filter is quickly dried in a desiccator in vacuum over $CaCl_2$ after some water of the filter is blotted off with special care and is kept intact for one day or so. This dry precipitate does not completely dissolve in redistilled water saturated with H_2. This solution gives a brilliant light if the luciferase is added but no more Millon or ninhydrin reaction. Moreover this dry precipitate completely dissolves in absolute methyl or ethyl alcohol. In this way some crystals of $(NH_4)_2SO_4$ which do not dissolve in alcohol are separated and filtered off. This alcohol solution is perfectly clear and is fairly stable. It gives a brilliant light if the luciferase is added but no Millon or ninhydrin reaction.[1] The results are summarized in table 1.

The luciferine is also slowly extracted by absolute propyl alcohol. The writer thought, therefore, that this alcohol might substitute for the action of $(NH_4)_2SO_4$ in dissolving the luciferine and in precipitating the substance or substances which give Millon and ninhydrin reactions. This is not, however, the case. The extract of absolute propyl alcohol still contains a substance or substances besides the luciferine as it gives a fair ninhydrin reaction but no Millon. It is noticeable, however, that a

[1] A preliminary note was published in the Japanese Journal of Zoölogy, in April, 1922.

pink color temporarily appears when Millon's reagent is added to it though it soon fades away. No color appears even though the mixture is heated.

The evidence from isolation experiments indicates that the luciferine differs from the substance or substances which give Millon and ninhydrin reactions. So far as the well known color reactions for proteins are concerned the luciferine seems not to be a protein or amino-acid because no ninhydrin reaction appears in the luciferine solution. The substance or substances which give Millon and ninhydrin reactions in the last isolation process of the present experiments may be protein derivatives, namely, peptones, even though no biuret is detected. The writer found that $(NH_4)_2SO_4$ is the only substance which can completely separate the luciferine from the substance or substances which give Millon and ninhydrin reactions, so far as his tested chemical substances are concerned. According to Harvey, the Cypridina luciferine which is extracted with boiling absolute (ethyl ?) alcohol is not precipitated on saturation with $(NH_4)_2SO_4$ (1, p. 282). But this seems to be an experimental error. The luciferine extracted with absolute ethyl alcohol, hot or cold, is completely precipitated on saturation with powdered $(NH_4)_2SO_4$.

SALTING OUT. The Cypridina luciferine isolated by the combination method of absolute methyl and ethyl alcohols and precipitated on saturation with powdered $(NH_4)_2SO_4$ and dried on the filter is incompletely dissolved in redistilled water saturated with H_2. Or it completely dissolves in absolute methyl or ethyl alcohol.

This aqueous solution of the luciferine contains some $(NH_4)_2SO_4$ and is unstable. So experiments should be conducted as quickly as possible. The luciferine solution forms no precipitate on saturation with powdered NaCl. The result is the same when the saturated luciferine solution with NaCl is acidified with HCl or acetic acid.

The luciferine solution added to an equal volume of the saturated solution with $MgSO_4$ or $(NH_4)_2SO_4$ forms no precipitate. The same is true when the half-saturated luciferine solution with $MgSO_4$ or $(NH_4)_2SO_4$ is acidified with HCl or acetic acid. The luciferine solution on saturation with powdered $MgSO_4$ causes no precipitate. The same is true when the luciferine solution saturated with $MgSO_4$ is acidified with HCl or acetic acid. As already stated, the luciferine solution on saturation with powdered $(NH_4)_2SO_4$ completely precipitates. In this respect the luciferine may be a kind of secondary proteose if it is assumed to be a protein or protein derivatives even though it gives no color reaction as a protein or amino-acid.

ALKALOIDAL REAGENTS. The aqueous solution of the luciferine in question is entirely useless for alkaloidal experiments because it is unstable. But the absolute methyl alcohol solution of the luciferine is fairly stable and was used for some experiments.

A small amount of phosphomolybdic acid solution added to the alcohol solution of the luciferine, since the luciferine is injured by its excess, forms no precipitate but a slight turbidity appears. If this turbid solution is acidified with dilute HCl solution it gives a slight precipitate. But no luciferine is detected in the precipitate or the filtrate indicating the loss of its luminous property.

A not excess solution of phosphotungstic acid added to the alcohol solution of the luciferine causes a slight precipitate which does not filter clear. The filtrate gives a bright light with the luciferase. If the filtrate is acidified with dilute HCl solution, it gives an abundant precipitate. The precipitate washed with very dilute phosphotungstic acid in alcohol on the filter does not completely dissolve and produces no light if the luciferase is added. The filtrate is perfectly clear and contains the luciferine as it produces a bright light with the luciferase. These results seem to indicate, therefore, that the luciferine is not precipitated by phosphotungstic acid + HCl. If a small amount of phosphotungstic acid is added to the filtrate, however, it causes a slight further precipitate, indicating some more substance left in it. Both the precipitate and the filtrate produce no light with the luciferase indicating the harmful effect of the excess phosphotungstic acid. These results obscure the question whether the luciferine is precipitated by phosphotungstic acid + HCl or not. So no definite conclusion is reached from these experiments. Absolute methyl alcohol extract of the luciferine which is evaporated to dryness on the water bath and is incompletely dissolved in redistilled water saturated with H_2 is completely precipitated by phosphotungstic acid as shown in table 1. In this aqueous solution, however, there is some substance or substances besides the luciferine as it is already proved. For this reason the precipitation experiments with phosphotungstic acid on the comparatively pure luciferine is very important to decide the very nature of the luciferine. Unfortunately however, the results of experiments are not conclusive, even though they seem to indicate that the luciferine is not precipitated by phosphotungstic acid.

Tannic acid in absolute ethyl alcohol added to absolute methyl or ethyl alcohol solution of the luciferine in question forms no precipitate but causes turbidity and the luciferine remains intact in the solution. If this turbid solution is acidified with dilute HCl, some precipitate forms. The precipitate washed with very dilute tannic acid in alcohol contains sometimes a trace of the luciferine which may be due to a not thorough washing, that is, the adhesion of the luciferine to the precipitate. The filtrate is clear and contains the luciferine as it produces a bright light with the luciferase. The addition of a small amount of tannic acid to the filtrate causes further precipitate. But this addition is harmful for the luciferine since neither the precipitate nor the filtrate produces light with the lucif-

erase. This result is quite similar to the case of phosphotungstic acid. In the first place, the writer thought that the luciferine in question which is dissolved in absolute methyl or ethyl alcohol may remain intact in the filtrate as tannic acid is also dissolved in absolute ethyl alcohol. The result does not come out as supposed.

HEAVY METAL SALTS. The addition of 1 per cent $HgCl_2$ drop by drop to 10 cc. of the aqueous solution the luciferine in question forms no precipitate. The same is true even though $HgCl_2$ + NaOH or acetic acid is added. The luciferine on addition of an excess of $HgCl_2$ loses the property of luminosity.

TABLE 2

Isolation process of the Cypridina luciferine

The (A) of table 1 precipitated by absolute ethyl alcohol

Precipitate gives Millon and ninhydrin reactions but no light (no luciferine)	Filtrate gives Millon and ninhydrin reactions and a brilliant light (luciferine). Evaporate the filtrate to dryness, dissolve in H_2O and saturate with $(NH_4)_2SO_4$

Precipitate which is indicated by (B) gives a brilliant light (luciferine) but no Millon or ninhydrin reaction	Filtrate gives Millon and ninhydrin reactions but no light (no luciferine)

Neutral Pb acetate added to the luciferine solution gives no precipitate. But Pb acetate + NaOH added to the solution gives a precipitate which filters clear. The clear filtrate produces a bright light with the luciferase. The precipitate washed on the filter and dissolved in water gives no light with the luciferase. This precipitate is not, however, necessarily due to the presence of the luciferine, because if a small amount of NaOH is added to the neutral Pb acetate solution without the luciferine it also causes a precipitate. Lead acetate + acetic acid added to the luciferine solution forms no precipitate.

The addition of $AgNH_3$ to the luciferine solution forms a slight precipitate but the precipitate does not filter clear. So the result is not decisive. The addition of $FeCl_3$, $ZnSO_4$, or $CuSO_4$ to the luciferine solution gives no precipitate.

There is no evidence, therefore, from experiments with heavy metal salts, to favor the idea that the luciferine is a protein.

ACIDS AND ALKALIS. The luciferine is not precipitated by HCl, H_2SO_4, HNO_3, acetic acid, or trichloroacetic acid. Also it is not precipitated by KOH, NaOH, or NH_4OH. All the results hitherto mentioned are summarized in table 1.

DISCUSSION. The Cypridina luciferine is soluble in water, is neutral in the aqueous solution, and is uncoagulable by heat. Judging from these characteristics of the luciferine, it is readily supposed without further experiments that the luciferine may be either a kind of proteose or peptone, if it is a protein or protein derivative. In this respect the saturation of the luciferine solution with $(NH_4)_2SO_4$ may be best to decide whether the luciferine is a proteose or peptone. Hawk gives warning that "the separation of proteoses and peptones by means of fractional precipitation with ammonium sulphate does not possess the significance it was once supposed to possess . . . ." (2, p, 120). But generally speaking, if the luciferine is precipitated on saturation with $(NH_4)_2SO_4$, it would be a kind of proteose, assuming it as a protein or protein derivative. If not, it would be a kind of peptone.

The writer's salting-out experiments show that the luciferine is completely precipitated on saturation with $(NH_4)_2SO_4$ but not with $MgSO_4$. Moreover it is not precipitated with half-saturated $(NH_4)_2SO_4$. In these respects the luciferine would be a secondary proteose. If it is a secondary proteose, however, it should be precipitated by $K_4Fe(CN)_6$ + acetic acid or by heavy metal salts, but it is not. The only result which is obtained with saturated $(NH_4)_2SO_4$ would not be enough to regard the luciferine as a protein or protein derivative.

Moreover the luciferine which is isolated with the writer's combination method gives no color reactions as a protein or protein derivative. In this respect the luciferine could not be a protein. No protein which does not give color reactions is known as yet, so far as the writer is aware. The luciferine could not be an amino-acid either, because amino-acids generally give ninhydrin reaction but the luciferine does not.

Unfortunately, the results of precipitation experiments with alkaloidal reagents are not all decisive. The question whether the luciferine is a protein or not, therefore, still remains undecided. It is worth while to mention that the luciferine solution gives the Molisch reaction for general carbohydrates. But redistilled water which the writer used gave always the Molisch reaction without the luciferine even though it was always distilled thrice.

SUMMARY

1. The luciferine of Cypridina hilgendorfii is soluble in water, is neutral in the aqueous solution and is uncoagulable by heat. From this nature,

it is readily supposed without experiments that the luciferine may be a kind of proteose or peptone, if it is assumed to be a protein.

2. But the luciferine is also soluble in absolute methyl, ethyl and propyl alcohols.

3. The luciferine is extracted with absolute methyl alcohol for six hours and the extract is evaporated to dryness on the water bath. The residue with the luciferine is preserved intact over three months if kept in a desiccator over $CaCl_2$. The residue dissolves in redistilled water saturated with H_2 forming an opalescent solution which produces a brilliant light with the luciferase.

4. This luciferine solution gives Millon, ninhydrin, and Molisch reactions but no biuret, xanthoproteic or tryptophane.

5. The luciferine in this solution is completely precipitated by phosphotungstic acid or on saturation with $(NH_4)_2SO_4$ but by no other chemicals, as the results are shown in table 1.

6. The absolute methyl alcohol extract of the luciferine is evaporated down to a "chyme" which gives a voluminous precipitate with absolute ethyl alcohol. The precipitate produces no light with the luciferase but it gives Millon and ninhydrin reaction. The filtrate, however, contains the luciferine as it produces a brilliant light with the luciferase and also gives Millon and ninhydrin reactions.

7. The filtrate mentioned the last is again evaporated to dryness on the water bath. The residue is dissolved in redistilled water saturated with H_2 forming an opalescent solution which produces a brilliant light if the luciferase is added. The luciferine in this solution completely precipitates on saturation with $(NH_4)_2SO_4$. The filtrate produces no light with the luciferase but it still gives Millon and ninhydrin reactions.

8. The precipitate mentioned the last is quickly dried in the vacuum desiccator over $CaCl_2$ and is dissolved in redistilled water saturated with H_2 or in absolute methyl or ethyl alcohol. This solution contains the luciferine as it produces a brilliant light with the luciferase but it gives no Millon or ninhydrin reaction. It gives Molisch's reaction.

9. The luciferine in this last aqueous solution is not precipitated by any other salts except on saturation with $(NH_4)_2SO_4$, nor by heavy metal salts, or acids and alkalies. The results obtained with alkaloidal reagents are not conclusive.

The work was supported by Mr. Jihachi Hamano.

BIBLIOGRAPHY

(1) HARVEY: Journ. Gen. Physiol., 1919, i, 269.
(2) HAWK: Practical physiological chemistry. 6th ed., 1918.
(3) KANDA: This Journal, 1920, l, 544.
(4) KANDA: This Journal, 1921, lv, 1.

FACTORS CONCERNED IN BLOOD VOLUME REGULATION[1]

ALFRED CHANUTIN, ARTHUR H. SMITH AND LAFAYETTE B. MENDEL

From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Connecticut

Received for publication February 4, 1924

The mechanism for the maintenance of water equilibrium in the body is concerned with the nutrition of the cell, the excretion of waste products and the regulation of the blood volume. The ability of the organism to adjust itself to alterations in the fluid intake and outgo is well recognized. The factors involved in the maintenance of fluid equilibrium, although of prime physiological importance, are by no means clear. The present investigation deals with the problem of the immediate fate of fluid introduced into the body under certain experimental conditions.

EFFECTS OF INTRODUCTION OF FLUID INTO THE BODY. Notwithstanding the classic paper of Cohnheim and Lichtheim (8), in which they showed that an excess of fluid, introduced intravenously, leaves the blood stream, little attention was directed to the regulation of the blood volume until Sherrington and Copeman (31), studying the specific gravity of the blood of rabbits after injections of saline solutions, found that the larger part of the fluid introduced disappeared from the blood stream during and immediately after the injection. The more recent work of Bogert, Underhill and Mendel (5), and Smith and Mendel (32) has demonstrated a rapid return of the blood volume, as indicated by its hemoglobin content, to normal after the injection of saline solutions of varied composition into anesthetized rabbits. Furthermore, it was pointed out by Smith and Mendel that a considerable fraction of the fluid introduced was left in the body even after the blood volume had returned to normal Precisely where this fluid remained was not determined.

Bayliss and Starling (4) showed that the arterial and portal blood pressures are increased in the early intervals after a rapid injection of a large quantity of saline solution. Under such conditions, resulting in an increased blood pressure in the intestinal and liver capillaries, Starling (35) found that the lymph flow from the thoracic duct increased.

In the course of the adjustment of blood volume, diuresis may occur. Magnus (23) noted in one case in a rabbit after injection of saline that the greatest flow of urine ensued after the blood pressure had returned to normal. In some of his experiments (24) there was no constant relationship between diuresis, arterial and venous blood pressures, and kidney volume. In one experiment Gottlieb and Magnus (14) found that although the blood pressure was lower than normal, there was a marked diuresis at the end of the injection.

[1] The experimental data in this paper are taken from the dissertation submitted by Alfred Chanutin in partial fulfilment of the requirement for the Degree of Doctor of Philosophy, Yale University, 1923.

Meek and Eyster (25) demonstrated that fluid equivalent to 30–100 per cent of the blood volume may be injected into anesthetized dogs without causing dilatation of the heart. Under such conditions there is no rise in arterial pressure commensurate with the quantity of the fluid injected.

From the experimental evidence at hand it appears that the vascular changes resulting from intravenous introduction of fluid are fairly well determined. The correlation of these changes with urine secretion is, however, obscure.

One might expect that when ingested fluids are absorbed a hydremia may ensue, and this in turn may lead to diuresis. Jones (17) in 1887 demonstrated a decrease in the specific gravity of the blood after 700 cc. of water had been drunk. Strauss and Chajes (36) noticed a slight dilution in the serum of several patients after water drinking. According to Engel and Scharl (12), patients with normal kidneys, who were given alkaline water to drink in amounts varying from 1000 cc. to 1400 cc., showed no blood dilution as determined by the refractive index. Haldane and Priestley (15) could not detect changes in the concentration of hemoglobin in the blood after drinking 5 liters of water over a period of several hours, although the rate of urinary secretion was increased to 1200 cc. an hour. This was confirmed by Adolph (1). After drinking water, Priestley (29) detected in the blood a small decrease in the electrical conductivity, however, apparently due to a drop in concentration of the salts of the blood. The electrical conductivity of the serum of diabetics was found by by Christie and Stewart (7) to be changed very little during thirst or periods of water intake.

In contrast to the results obtained with water, the ingestion of saline solutions causes a slight decrease in the hemoglobin concentration of the blood, with an accompanying diuresis (29), (1). In infants, the oral administration of water or salt solution may cause a marked hydremia; where water is used there is no diuresis, and the experiments with saline show oliguria (2).

It thus appears that attempts to induce hydremia by the oral administration of fluids have apparently not given consistent results. Explanations suggested for the diuresis observed are far from satisfactory, since experimental evidence has failed to correlate the flow of urine with the degree of hydremia produced.

EXPERIMENTAL OBSERVATIONS. *The procedure adopted.* Carefully selected, healthy bitches were used in these experiments. They received a meal of meat and dog biscuit once daily; water was given *ad libitum.* During the experimental period, the unanesthetized animal was strapped on its back, as much freedom of movement being allowed as was feasible. The animal became so accustomed to this procedure that restlessness or struggling was rarely encountered. In fact, injections into or withdrawals from the jugular could be accomplished without difficulty by one experimenter, unaided.

The relative blood volume was determined by a comparison of the percentage of the hemoglobin after the administration of fluids with that before the administration. An illustration of the calculation follows: taking the original blood volume as 100 per cent, and the original content of hemoglobin as 90 per cent before the injection and 60 per cent after injection, the relative blood volume after injection would be $\frac{90}{60} \times 100 = 150$.

Blood samples for the estimation of hemoglobin were taken from the ear veins. It was found that such samples gave the same values as the blood removed directly from the jugular vein. Only a few drops of blood were necessary for the determination. Larger samples for the estimation of blood solids were obtained from the jugular, by means of a hypodermic needle and syringe. Hemoglobin determinations were made according to the method suggested by Newcomer (26). Urine was obtained by catheterization. In calculating the extent to which the introduced fluid was recovered in the urine, no correction has been made for the normal output of urine.

The use of hemoglobin as a valid index of the blood volume changes has been justified by previous workers (5), (20).

Diurnal variations of blood volume. Variations in the concentration of the blood of the normal organism, from day to day and also within a single day, have been reported by a number of investigators (31), (27), (30), (33), (11). An attempt was made to determine whether the content of hemoglobin in the blood of normal, unanesthetized animals varied as a result of other than the experimental conditions already outlined. In table 1 it may be seen that fluctuations in the hemoglobin concentration of the blood occur during the day. Under these conditions it was found that the total solids of the plasma were not affected. It is apparent that the degree of change in the content of the hemoglobin is variable in the course of the day. Furthermore, it will be seen that the length of time during which these changes may persist cannot be predicted. Of course, such incidental variations in the concentration of the blood in the normal unanesthetized animal should be taken into account in a consideration of the changes in the blood volume under the experimental conditions mentioned. The physiological factors re-

TABLE 1

Diurnal variations in the blood volume of normal, unanesthetized animals; relative blood volume

TIME AFTER BEGINNING EXPERIMENT	DOG				
	1	1	2	4	5
hours					
0	100	100	100	100	100
1	104	100	110	108	96
2	105	100	110	108	100
3	114	101	114	111	107
4	114	101	114	107	109
5	114	101	114	100	
6	117	109	114	114	
7	102	121			

sponsible for fluctuations in the concentration of the blood in the resting organism without food are not at present known.

Influence of anesthetics. Inasmuch as most of the investigations dealing with this problem have been carried out on animals under anesthesia, it seems advisable to discuss the possible effects of anesthetic drugs on the organism. "There is a group of substances (narcotics and anesthetics) which act on the living cells in such a way as to abolish temporarily those activities which we regard as manifestations of life" (3). Under these conditions, the organism may not always react normally to physiological stimulation.

Frey (13) demonstrated that diuresis after the ingestion of water is prevented by morphine, urethane, chloral hydrate, or ether. He showed further that the diuresis after intravenous injections of saline is not affected by the same narcotics. It was noted by MacNider (21) that anuria occurred in normal dogs under surgical anesthesia induced by ether or chloroform. The anuria resulting from ether was overcome after saline was injected intravenously. Chloroform, on the other hand, caused a more marked anuria and the injection of saline was of no value in reëstablishing the urine flow.

Boycott and Price-Jones (6) observed that the restoration of the blood volume after hemorrhage is less complete in anesthetized than in the unanesthetized rabbit. Furthermore, the time required for the return of the blood volume to normal after plethora, was longer in the unanesthetized than in the anesthetized animal. The authors attributed the differences described to a change in the permeability of the capillary wall by the anesthetics. Bogert, Underhill and Mendel (5) noted, with respect of saline injections, that morphine sulphate injected subcutaneously in large doses into dogs, produced a delayed regulation of the blood volume, comparable with that resulting from a condition of nephritis.

It appears from such investigations that anesthetics and narcotics may impair the functional processes of the organism. For this reason, the experimental work in this investigation was carried out, for the most part, on unanesthetized animals. Where anesthesia was employed it was kept light by means of an ether insufflation apparatus. During the entire period of experimentation the animals were kept warm. Struggling was encountered during the first stage of anesthesia when the ether was administered by means of a cone.

Ether anesthesia has a definite effect upon the concentration of the blood. Within a period of one to three minutes the concentration of the hemoglobin in the blood was found, in two experiments, to be increased from 86 to 108 per cent and from 93 to 110 per cent (table 2). Furthermore, prolonged anesthesia for four hours seemed to cause an increase in the concentration of the blood instead of a gradual fall as hitherto reported. After the ether is discontinued and the animal has regained consciousness the blood volume still remains low. The urinary secretion during this period is diminished; no complete anuria, however, was observed.

Oral administration of water and physiological saline. A definite dilution of the blood is obtained after the ingestion of large amounts of water

(100 cc. per kgm.). Smaller quantities of water (50 cc. per kgm.) cause only a very slight increase in the relative blood volume. Saline solutions, however, produce a more marked hydremia than water. In contrast to the dog, human subjects can tolerate only a relatively small quantity of water. Whereas a 10-kilo animal can be given a liter of water during a period of ten minutes, it would be impossible for a man weighing 70

TABLE 2

The effect of ether anesthesia upon the concentration of the blood of normal dogs

TIME AFTER BEGINNING ANESTHESIA		HEMOGLOBIN	RELATIVE BLOOD VOLUME	REMARKS
Bitch 3, weighing 10.4 kgm.		Light anesthesia maintained throughout the experiment		
	minutes	*per cent*		
Normal	8:45 a.m.	96		
Normal	9:30 a.m.	86	100	
	9:34 a.m. Anesthesia begun			
1	9:35	89	96	Struggling
3	9:37	108	80	No struggling; reflex
5	9:39	107	80	No reflex
30		114	75	Reflex
60		112	76	
90		108	79	
120		110	77	
180		110	77	Ether stopped
210		118	73	
Bitch 7, weighing 11.0 kgm.		Light anesthesia maintained throughout the experiment		
Normal	8:50 a.m.	94		
Normal	9:35 a.m.	93	100	
	9:37 a.m. Anesthesia begun			
1	9:38	110	85	Struggling
3	9:40	110	85	No reflex
60		120	78	Irregular anesthesia
90		118	79	
120		122	76	
180		120	78	
240		118	79	Ether stopped
300		118	79	
360		120	78	Animal fully conscious, but walking is incoördinated

kgm. to take 7000 cc. in the same time in order to obtain comparable conditions. In experiments in which a maximum quantity of water was ingested by man, it has been found that the hemoglobin concentration was not changed (15).

Evidently there is a dilution of the blood shortly after the oral administration of fluids, indicating a prompt absorption from the intestinal

tract. Despite the hydremia present during the first half hour after the introduction of water or saline by mouth, there is no appreciable change in the rate of the secretion of urine within that period. The greater part of the ingested fluid is subsequently secreted by the kidneys during the first few hours. It should be emphasized that renal activity is not dependent on the degree of hydremia. Data from some typical protocols are given in table 3.

TABLE 3

Water and saline given orally

TIME AFTER INGESTION	HEMOGLOBIN	RELATIVE BLOOD VOLUME	AMOUNT OF FLUID RECOVERED IN URINE	
Bitch 6, weighing 7.8 kgm. Oral administration of 780 cc. of water (100 cc. per kgm.) in seven minutes				
minutes	*per cent*		*cc.*	*per cent*
Normal	94	100	0	
After water				
30	85	111		
60	79	119	98	13
90	76	124		
120	80	117	176	35
180	85	111	146	54
210	78	120		
240	78	120	109	68
300	80	117	88	79
			617	
Bitch 8, weighing 8.0 kgm. Oral administration of 800 cc. of saline (100 cc. per kgm.) in ten minutes				
Normal	91	100	0	
After saline				
30	74	123		
60	66	137	56	7
90	63	144		
120	63	144	278	42
180	74	123	143	59
240	72	126	61	67
300	82	111	45	73
			583	

Intravenous injection of physiological saline. Inasmuch as there are few accounts of the regulation of blood volume after the injection of saline into unanesthetized dogs, it seemed worth while to try the effect of saline introduced in different amounts and at different rates. Sodium chloride solution (0.92 per cent) warmed to body temperature was injected into the jugular vein. In the case of *rapid* injections, a period varying from

5 to 15 minutes was required; for the slow injections, the time of introduction depended on the amount of fluid used, an attempt being made to simulate the presumably *slow* rate at which fluid enters the blood stream by intestinal absorption. Toxic symptoms due to the injection were never noted. Occasionally, an animal would vomit a small amount of a mucous-water mixture several hours after a rapid injection. Diarrhea during or after the experimental period never occurred.

There was little or no hydremia during or after the slower injections (table 4). It was further noted that a marked diuresis occurred without any indications of hydremia.

Experimental evidence obtained with anesthetized animals by other workers has shown that the time required for the return of the blood volume to normal is quite constant after rapid injections of the same quantities of saline solution. In the writers' experience on unanesthetized dogs, however, the time required by the organism to eliminate injected saline from the blood stream varies considerably. After a rapid injection of 100 cc. of saline per kilo of body weight, for example, periods varying from one to four hours elapsed before the hemoglobin had approximated or attained the normal. Usually, however, the adjustment occurred within two hours as shown in table 5.

The greater part of the fluid introduced leaves the circulation during and immediately after the injection. At this period, none of this fluid can be accounted for in the urine. There were marked variations in the rate of urinary secretion. A volume of urine equal to the amount of fluid injected was rarely obtained within the time in which the blood was returning to its normal concentration. This observation, that the blood is practically free of the excess of injected fluid long before the latter is excreted, together with the fact that a relatively large amount of fluid may be retained somewhere in the body, furnished the occasion for studying the possible temporary fluid reservoirs.

The anesthetized dog is able to restore its blood volume after saline injection as well as the unanesthetized animal. Under normal conditions, the two animals used in these experiments had eliminated 67 and 59 per cent, respectively, of the fluid injected intravenously (100 cc. per kgm.) at the end of two hours when the blood volume had returned to normal. Under ether anesthesia, however, the fluid recovered in the urine under the above conditions amounted to 34 and 43 per cent, respectively. The fact that these animals under ether anesthesia were able completely to restore the blood to its normal concentration, although the rate of urinary secretion was diminished, would seem to indicate further that *the kidney is not essential for the immediate regulation of blood volume.*

It has thus been shown that the rate of disappearance of water from the blood stream depends somewhat on the path and rate of introduction.

TABLE 4

Experiments showing the regulation of blood volume and rate of urinary secretion after slow injections of saline into unanesthetized animals

TIME INTERVAL	INJECTED 100 CC. PER KGM. AT RATE OF 10 CC. PER MINUTE		TIME INTERVAL	INJECTED 50 CC. PER KGM. AT RATE OF 5 CC. PER MINUTE		TIME INTERVAL	INJECTED 25 CC. PER KGM. AT RATE OF 2.5 CC. PER MINUTE	
	Relative blood volume	Fluid recovered in urine		Relative blood volume	Fluid recovered in urine		Relative blood volume	Fluid recovered in urine
minutes		*per cent*	*minutes*		*per cent*	*minutes*		*per cent*
Before injection	100		Before injection	100		Before injection	100	
During injection			During injection			During injection		
20	100	4				20	105	
40			40	100		40	106	
60	107	13						
80	108	29						
117	107	44	88	104	15	62	114	3
After injection			After injection			After injection		
30	107	64	30	91	69	30	108	
60	101	80	60	93	85	60	101	33
120	101	97	120	100	88	120	101	58
180	99	103	180	101	91	180	99	67
240	108	105	240	95	92	240	96	76

TABLE 5

Typical experiments illustrating the regulation of blood volume and rate of urinary secretion after rapid intravenous injection of saline solution into unanesthetized animals

TIME INTERVAL	100 CC. PER KGM. BODY WEIGHT		50 CC. PER KGM. BODY WEIGHT		25 CC. PER KGM. BODY WEIGHT	
	Relative blood volume	Fluid recovered in urine	Relative blood volume	Fluid recovered in urine	Relative blood volume	Fluid recovered in urine
minutes		*per cent*		*per cent*		*per cent*
Normal	100		100		100	
After injection						
Immediately	157		133		132	
30	117	20	112	7	106	3
60	117	34	107	13	111	4
120	106	57	105	32	106	7
180	106	76	102	46	111	9
240	106	82	100	57	100	15

Fluids given in sufficient amounts *by mouth* may induce a hydremia accompanied by a diuresis. *Slow intravenous injections* insufficient to produce hydremia are nevertheless followed by a marked diuresis. The rate of a reëstablishment of a normal blood volume after the *rapid* injection of sodium chloride solution depends on the amount injected; the recovery is slow with larger amounts, and relatively fast when smaller volumes are introduced. *Ether anesthesia* does not delay the return of the blood volume to normal after the rapid injection of saline. The present observations confirm anew the conclusions that *hydremia is not the foremost or at least the only factor that initiates diuresis.*

THE FATE OF FLUIDS INJECTED INTRAVENOUSLY. From the preceding experiments it is obvious that injected saline solution leaving the blood stream does not entirely disappear promptly from the body through the activities of the kidneys. Therefore, an excess of fluid must remain either in the body cavities, gastro-intestinal tract, lymph vessels, or "tissues." Previous investigators have presented evidence regarding the probable depots for the transitory excess of fluid in the body, but there is a difference of opinion in regard thereto.

Cohnheim and Lichtheim (8) demonstrated that large quantities of fluid could be accommodated by an animal before death occurred. Five to six times the blood volume was injected into a dog within a half-hour without causing threatening symptoms, and in one case death did not occur until saline equivalent to 92 per cent of the body weight was injected. During the infusion, the changes in the content of solids of the blood showed that a considerable portion soon left the vessels. It was found that the extreme hydremia was associated with ascites, edematous swelling, and watery evacuations from the intestines. The lymph flow from the thoracic duct was immediately increased, and became still greater after subsequent injections until the fluid poured out in jets. Despite this enormous increase in lymph originating from the abdominal organs, it was interesting to note that the lymphatics of the extremities did not yield any more fluid than those of the normal animal. Autopsies after these experiments showed that the abdominal cavity and its organs were markedly edematous. The stomach and intestines were regularly found to be distended with fluid. No fluid was found in the thoracic cavity, however, and there was no edema of the muscular or subcutaneous tissue, or of the central nervous system and its membranes. Cohnheim and Lichtheim were of the opinion that the veins, with their elastic and easily distended walls, must contain a large amount of the excess of fluid.

Dastre and Loye (10) injected large quantities of 0.7 per cent sodium chloride solution into unanesthetized rabbits. After a time an equilibrium tended to be established and almost as much fluid escaped by the kidneys as was being injected. They found that during the injection and for some time afterward, 25 per cent of the fluid remained in the blood and 75 per cent went into the serous cavities and tissues. Autopsies performed 24 hours after death showed that there was fluid in the pleural and abdominal cavities, and that the cecum was markedly distended by fluid, but that the small intestine and stomach were in normal condition.

The factors concerned in diuresis and hydremia were studied by Magnus (23). He calculated that after injections of isotonic saline, the greater part of the fluid

entered the tissues. He believed that the tissues act as a depot for absorbing and disposing of fluid.

In 1904, Engels injected more than a liter of 0.6 per cent saline, on the average, into dogs, and calculated that the muscles took up 68 per cent, the skin 18 per cent, and the remainder of the body 14 per cent of the surplus of fluid stored.

Sherrington and Copeman (31), and Bogert, Underhill and Mendel (5) excluded the kidneys from the circulation before the intravenous injection of saline, and found no difference in the speed of return of the specific gravity or hemoglobin content of the blood to normal. In order to exclude the capillaries of the limbs as a channel of escape of injected fluid, Sherrington and Copeman ligated the blood vessels of the extremities, but even this did not affect the return to normal of the specific gravity after injection. Autopsies revealed fluid in the peritoneal cavity, and to a lesser extent in the pericardial and pleural cavities. No subcutaneous or muscular edema was noted. The intestines were found to be distended by a thin watery fluid.

It was demonstrated by Magnus (22) that slow injections of large quantities of saline into rabbits and dogs do not cause skin edema. But after poisoning with arsenic, chloroform, chloral hydrate and, in the case of the dog, phosphorus, or after the extirpation of the kidneys, or the tying of the ureters, these large infusions caused skin edema. According to Magnus, the drugs caused an increased permeability of the capillaries.

Smith and Mendel (32) could not account for all of the fluid which leaves the circulation in the restoration of blood volume after intravenous injection of saline, either in the muscle or edema fluid. They suggested that, in rabbits, the fluid secreted into the gastro-intestinal tract accounted for a considerable part of the "lost" water.

It was noted by Johansson and Tigerstedt (16) that the liver always became enlarged after transfusions of blood or saline. They stated that after the transfusions of large amounts of fluid, the liver becomes "bretthart." If it is cut out from the body of the animal after death, a large quantity of fluid flows out. According to them an appreciable amount of fluid is taken up from the blood stream and is held by the liver.

The investigations of Lamson and Roca (19) proved that physiological saline (25 cc. per kgm. body weight) injected intravenously into normal dogs under ether anesthesia, leaves the blood stream in one-half hour. When an Eck fistula was made, thus eliminating the liver, and saline was injected, the return to normal was slower, approximating two hours. A series of instantaneous x-rays taken of dogs under ether during the injection of saline showed a definite increase in the size of the liver.

By means of photomicrographs of the vessels of the dog's ear, Meek and Eyster (25) demonstrated that new capillaries were opened after large injections of saline, gum acacia, or blood. These authors stated: "We feel confident, then, that the heart is not dilated by the injections even of large quantities of fluid into the circulation because the excess is taken care of in the capillaries and venules." From postmortem evidence, they believed the capillaries of the liver and lungs were reservoirs for the excess of fluid.

As has already been noted, rapid injection of saline into dogs causes a rise in the pressure in the portal vein and vena cava, thus giving evidence of increased pressure in the intestinal and hepatic capillaries (4).

It was first shown by Worm-Müller (37) that large quantities of blood can be transfused into an animal without raising the blood pressure correspondingly. The failure to find this surplus of blood constituents in the lymph, tissues, or serous cavities led him to believe that the excess was disposed of in the capillaries and veins, especially of the abdominal organs. After transfusion, they were decidedly hyper-

emic, while the extremities, skin, subcutaneous tissue, and central nervous system showed no vascular fullness.

The importance of the capillaries as a storehouse for either red cells or plasma was suggested by Cohnstein and Zuntz (9). Vasomotor influences upon the capillaries were found to affect the blood concentration; constriction caused an increase, and dilatation a decrease, in the number of red cells

From the above discussion, it might be concluded that injected fluid can be stored temporarily in many places. Experimental evidence has been interpreted in the literature to show that the veins, muscles, skin, peritoneal cavity, intestinal tract, liver, capillaries, and "tissues" may act as reservoirs for the surplus to fluid.

EXPERIMENTAL OBSERVATIONS. *The water content of the muscles and liver after infusions of fluid into the circulation.* A consideration of the possible tissue reservoirs in the body naturally leads one, from a purely anatomical viewpoint, to the muscles and the liver. The former comprise about 40 per cent of the body weight, while the latter is the largest and the most vascular individual organ. Evidence presented by previous workers has been supposed to show that the muscles and liver may act as reservoirs for excess of water. An attempt has been made in the present investigation to determine by direct analyses the water content of the tissues considered.

The experiments on *muscle* were conducted under anesthesia so that comparable samples for the determination of the water content could be obtained from each animal before and after the introduction of fluids. Corresponding portions of the gastrocnemius muscle were excised from one hind leg before the injection, and from the other leg several hours thereafter, for analysis. It should be emphasized that, due to the anuria caused by the ether anesthesia, an unusually large amount of the injected fluid remained in the body at the time when the animals died. From table 6 it will be seen that the percentage of water in the muscle remains the same before and after injection of saline, thus eliminating this tissue as a possible reservoir.

Evidence obtained from autopsy and x-ray studies has led several investigators to consider the *liver* as the main depot for surplus of fluid in the body. In order to obtain further evidence on this question, direct measurements of the volume of blood and water in the livers of normal and injected animals were made.

The animals were killed at the desired time by an injection of air into the jugular vein. The abdominal and thoracic cavities were opened and the hepatic vessels ligated. The liver could then be excised without loss of blood. After removing the gall bladder the liver was weighed and ground to a hash. Under constant stirring, samples for total solids were taken. The remainder of the hash was extracted with several liters of

water and the hemoglobin determined therein by the colorimetric method depending on the principle of Palmer (28). From the data obtained the total blood volume and the water in the liver, other than that present in the blood, were calculated. A sample protocol is given below:

TABLE 6

Comparison of blood concentration, urine output, and water content of muscle before and after the injection of saline into anesthetized animals

BODY WEIGHT	TIME AFTER INJECTION	RELATIVE BLOOD VOLUME	WATER CONCENTRATION OF THE BLOOD	WATER CONTENT OF GASTROCNEMIUS MUSCLE	FLUID RECOVERED IN URINE	
kgm.	*minutes*		*per cent*	*per cent*	*cc.*	*per cent*
11.2	Before	100	77.9	74.8		
Injection of 1120 cc. of saline in nine minutes						
	30	124			53	5
	60	118	79.8		7	5
	90	118			6	6
	120	120	79.8		9	7
	180	116	79.7		28	9
	210	108	78.0		10	10
					—	
	210	Killed		75.1	113	
9.3	Before	100	78.6	74.6		
Injection of 930 cc. of saline in nine minutes						
	30	148			56	6
	60	145	82.1		36	10
	90	135			18	12
	120	137	81.2		10	13
	180	135			2	13
	200	135	81.2		3	14
					—	
	200	Killed		75.4	125	
5.5	Before	100	74.5	73.0		
Injection of 550 cc. of saline in five minutes						
	60	136			9	2
	90	136	79.0		6	3
	120	125			5	4
	180	139			1	4
	190	139	79.1		0	4
					—	
	190	Killed		72.2	22	

Bitch weighed 10.7 kgm. At 1 o'clock the hemoglobin of the jugular blood was 81 per cent, which is equivalent to 11.3 grams hemoglobin in 100 cc. of blood. The animal was killed at 1:09 by injecting air intravenously. The abdomen was opened, the vessels to the liver were ligated, and this organ was removed without loss of blood. The liver minus the gall bladder weighed 536 grams, or 5 per cent of the body weight. The liver pulp and the blood therefrom were stirred continually and total solid

samples were obtained. Five liters of distilled water were used to extract the hemoglobin from the hash. A portion of this extract was centrifuged to get rid of the turbidity; its hemoglobin was determined by Palmer's method. A standard was made by diluting 1 cc. of blood (81 per cent Hb.) to 250 cc. with 0.4 per cent ammonium hydroxide; the extract diluted five times was found to contain 1.66 times as much hemoglobin as the standard. The total solids of the blood and liver amounted to 24.8 per cent, and average of four determinations 24.9, 24.8, 24.8 and 24.8 per cent, so that the 536 grams of liver contained 403 grams of water. The latter, plus the fluid used in the extract (5000 cc.), equals 5403 cc., which in turn were diluted five times, bringing the total up to 27,015 cc. Since 1000 cc. of the standard contained 0.452 gram hemoglobin, the total extract contained $0.452 \times 27.01 \times 1.66 = 20.25$ grams hemoglobin. This is equivalent to $\frac{20.25}{11.3} = 178$ cc. blood in the liver. Five hundred thirty-six grams liver minus 178 grams blood = 358 grams of blood-free liver tissue. Further, 178 grams blood $\times$ 80 per cent water content equals 142.4 cc. water in the blood. Therefore, 403 grams water present in the liver minus 142.4 grams blood-water equals 250.6 grams water in the blood-free liver, or $\frac{250.6}{358} = 70$ per cent water in the blood-free liver.

Additional data concerning the blood and water content of livers before and after saline injections are given in table 7.

It has been assumed, in the calculations given above, that the blood of the liver has the same hemoglobin concentration as the blood in the jugular vein, and that the liver tissue itself contained very small amounts of hemoglobin. The first assumption is justified according to the observations of Krogh (18) who has pointed out that the "vaso serosa" of Cohnstein and Zuntz, and the still spaces for plasma, as postulated by Smith, Arnold and Whipple (34), do not exist but that there is an even distribution of cells and plasma in the vessels and capillaries of the body in the normal organism. The liver tissue contains only a very small amount of hemoglobin, according to Smith, Arnold and Whipple (34).

Autopsies performed on our animals several hours after the injection of saline have shown only a few cubic centimeters of fluid free in the peritoneal cavity. The liver was always enlarged. The stomach and intestines were found to contain only a small quantity of fluid. It was always noted that the fat tissue surrounding the mesenteric vessels had a pearly appearance due to the infiltration of water. The pancreas was moderately edematous. Water was never observed in the thoracic and pericardial cavities. The lungs were only slightly edematous. Edema of the skin or muscles was never seen. By a process of exclusion, the abdominal cavity and its organs must play the major rôle in the storage of water in the body.

From the experimental evidence already given, it appears that the dog's muscular tissue does not act as a reservoir for excess of fluid. Furthermore, determinations of the water content of the liver tissue indicate that

the enlargement of the liver is not due to an absorption of water but to an increased amount of blood. The fate of the fluid that disappears in large amounts from the blood vessels under the conditions discussed still remains to be ascertained.

Discussion. A discussion of the fate of fluid introduced into the body is likely to be replete with hypotheses, for thus far no definite comprehensive explanation has been satisfactorily advanced. In consideration of the conflicting evidence obtained by previous investigators, certain lines of attack have been suggested and, with conditions maintained constant, certain significant facts have been established in the present investigation.

It is unquestionably advantageous to carry out experimental work under conditions as nearly normal as is possible. In these experiments, such interfering factors as anesthesia, struggling, and larger operations have

TABLE 7

The blood and water content of livers before and after saline injection

DOG	BODY WEIGHT	LIVER WEIGHT	LIVER WEIGHT TO BODY WEIGHT	AVERAGE TOTAL SOLIDS OF LIVER	CALCULATED BLOOD VOLUME IN LIVER	WATER IN BLOOD-FREE LIVER	AMOUNT OF SALINE INJECTED	NORMAL HEMOGLOBIN	HEMOGLOBIN TWO HOURS AFTER INJECTION	FLUID RECOVERED IN URINE	FLUID RETAINED IN THE BODY
					Control animals (uninjected)						
no.	*kgm.*	*gm.*	*per cent*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*cc.*
9	10.7	586	5.5	23.7	158	**74**					
4	10.7	536	5.0	24.8	178	**70**					
					Animals injected						
8	9.4	679	7.2	26.7	224	**70**	940	114	93	524	416
5	8.6	593	6.9	22.7	188	**74**	860	78	73	542	318

been for the most part eliminated. But even so, we are confronted with variations in the concentration of the blood. These changes occur at irregular intervals in the same animal on different days and apparently are not due to any changes in rate of urinary secretion, to digestion or to any emotional changes, because the various factors mentioned have been carefully controlled.

Since the greater part of the experimental work carried out on this problem by previous investigators has been conducted on animals under ether anesthesia, it became important to know how this affects the concentration of the blood. There is little known concerning the action of ether on the concentration of the red cells of the blood and the information available is confusing. It appears from this investigation that ether causes a marked increase in the percentage of hemoglobin of the blood, which is

not only prolonged during the anesthesia but persists after consciousness has been regained. Therefore such a change cannot be entirely due to the effects of fright and struggling, but presumably is caused by the action of the anesthetic on the mechanism for the regulation of the blood volume. In all probability lack of oxygen, salivation and hyperpnea may be ruled out as factors in the phenomenon noted. It is apparent, therefore, that the normal physiological processes are affected by anesthesia, and furthermore, unanesthetized animals are to be preferred for experimental work whenever it is possible.

The present study has shown that when water is given to unanesthetized dogs, in massive doses per os within a short period, an unmistakable dilution of the blood is observed which may last for several hours although most marked at about the end of an hour after the administration of the fluid. It is worthy of note that during the first half hour after the ingestion of water by dogs, there is slight, if any, diuresis. Furthermore, the diuresis is not any more marked during the height of dilution of the blood than when the relative blood volume is nearly back to normal. A failure to secure a similar increase in the blood volume in man is due, no doubt, to the inability to ingest large enough quantities of water. In dogs, the amounts of water comparable to the supposedly large doses hitherto taken by man, do not cause any appreciable dilution of the blood.

When normal saline is given in the same way, the degree of blood dilution is more pronounced and more prolonged; although at the end of an hour, when compared to water introduced in a similar way, the rate of urinary secretion is decidedly slower. It is apparent, therefore, that the degree of hydremia under these conditions is not necessarily paralleled by an increase in the rate of kidney secretion.

The high blood dilution and the slow rate of urinary secretion after rapid intravenous injections on the one hand, and the negligible blood dilution with the marked diuresis after slow injections on the other hand, seem to fortify the above contention. Again we are challenged to find a working hypothesis for kidney action under these conditions. The difference in the degree of hydremia observed between oral administration and slow intravenous injection may result from the adjustment necessary in the process of diffusion through the gut wall or in the passage of the fluid through the portal and hepatic vessels.

Although there are plenty of references in the literature to localities in the body in which introduced fluid may collect, the conditions under which the experiments referred to therein were performed and the procedures to which the animals were subjected make it practically impossible to accept the conclusions arrived at. Especially does this seem justified in the light of the results of the present investigation. It has been emphasized that part of the fluid introduced intravenously may remain in the

blood stream for several hours, that when the blood volume has become approximately normal the excreted urine accounts for only part of the injected fluid, and that neither the muscles nor the liver hold unusual amounts of water at this stage.

It is possible that the water is distributed temporarily to every tissue in the body in such small amounts that our method of analysis cannot detect them. Many investigators have demonstrated that such a mechanism is unlikely. Again, after saline injection, the long tortuous intestine undoubtedly contains more fluid than it normally does both in the lumen and in the walls. It is probable from experimental and autopsy observations that the abdominal area and especially the lymph vessels comprise the "water reservoir" of the body. The present investigation has eliminated some of the possible "depots." The temporary storehouse for fluid in the body remains still to be found.

SUMMARY

Diurnal variations in the concentration of the hemoglobin of the blood occur in normal unanesthetized dogs kept in a state of muscular rest.

The administration of ether to an animal is followed within a few minutes by an increase in the percentage of hemoglobin in the blood; this condition is maintained during the anesthesia and for some time thereafter.

The rapid oral administration of *water* (100 cc. per kgm. body weight) causes an appreciable hydremia and diuresis which occurs soon after the ingestion; smaller quantities of water (50 cc. per kgm. body weight) have only a very slight effect on the concentration of the blood.

The oral administration of *saline solution* is followed by a more marked hydremia than results from the ingestion of equal volumes of water.

Saline solutions injected intravenously at a slow rate so as to simulate the rate of intestinal absorption cause no appreciable alteration in the concentration of the blood.

The rate of restoration of the blood volume after the rapid injection of saline depends primarily on the amount introduced. After large quantities (100 cc. per kgm. body weight) the time required for the return of the blood volume to normal varies considerably but in general this occurs in about two hours on the dog. With smaller quantities of saline the volume is restored much sooner.

When saline is injected into animals under ether anesthesia, the time required for the blood volume to return to normal is the same as in the unanesthetized animal; however, the rate of urinary secretion is diminished under the influence of the anesthesia.

The activity of the kidney is not essential for the immediate regulation of the blood volume after the intravenous injection of saline, since restoration of the volume is effected before the injected fluid leaves the body

through the urine. There must, therefore, be a temporary reservoir in the body for taking care of this excess of fluid.

Analyses of a typical muscle and of the liver tissue show that, within the limits of accuracy of the methods available, these tissues under normal conditions do not act as depots for appreciable volumes of the fluid introduced.

Part of the expenses of this study was defrayed by a grant from the Russell H. Chittenden Fund for Research in Physiological Chemistry.

BIBLIOGRAPHY

(1) Adolph: Journ. Physiol., 1921, lv, 114.
(2) Bakwin: This Journal, 1922, lx, 343.
(3) Bayliss: Principles of physiology. London, 1920, 138.
(4) Bayliss and Starling: Journ. Physiol., 1894, xvi, 159.
(5) Bogert, Underhill and Mendel: This Journal, 1916, xli, 189.
(6) Boycott and Price-Jones: Journ. Path. and Bacteriol., 1922, xxv, 335.
(7) Christie and Stewart: Arch. Int. Med., 1922, xxix, 555.
(8) Cohnheim and Lichtenheim: Arch. f. path. Anat. u. Physiol., 1877, lxix, 106.
(9) Cohnstein and Zuntz: Arch. f. d. gesammt. Physiol., 1888, xlii, 303.
(10) Dastre and Loye: Arch. de physiol. norm. et pathol., 1888, 4th series, ii, 93.
(11) Dreyer, Bazett and Pierce: Lancet, 1920, cxcix, 588.
(12) Engel and Scharl: Zeitschr. f. klin. Med., 1906, lx, 225.
(13) Frey: Arch. f. d. gesammt. Physiol., 1907, cxx, 66.
(14) Gottlieb and Magnus: Arch. f. exper. Path. u. Pharm., 1901b, xlv, 248.
(15) Haldane and Priestley: Journ. Physiol., 1915–1916, l, 296.
(16) Johansson and Tigerstedt: Skand. Arch. f. Physiol., 1889, i, 331.
(17) Jones: Journ. Physiol., 1887, viii, 1.
(18) Krogh: The anatomy and physiology of capillaries. New Haven, 1922, 67.
(19) Lamson and Roca: Journ. Pharm. Exper. Therap., 1921, xvii, 481.
(20) Lamson and Rosenthal: This Journal, 1923, lxiii, 358.
(21) MacNider: Journ. Pharm. Exper. Therap., 1920, xv, 249.
(22) Magnus: Arch. f. exper. Path. u. Pharm., 1899, xlii, 250.
(23) Magnus: Arch. f. exper. Path. u. Pharm., 1900a, xliv, 68.
(24) Magnus: Arch. f. exper. Path. u. Pharm., 1900b, xliv, 396.
(25) Meek and Eyster: This Journal, 1922, lxi, 186.
(26) Newcomer: Journ. Biol. Chem., 1919, xxxvii, 465.
(27) Oliver: Lancet, 1896, January–June, 1699.
(28) Palmer: Journ. Biol. Chem., 1918, xxxiii, 119.
(29) Priestley: Journ. Physiol., 1915–1916, l, 304.
(30) Rabinowitch: Journ. Lab. Clin. Med., 1923, ix, 120.
(31) Sherrington and Copeman: Journ. Physiol., 1893, xiv, 52.
(32) Smith and Mendel: This Journal, 1920, liii, 323.
(33) Smith: This Journal, 1920, li, 221.
(34) Smith, Arnold and Whipple: This Journal, 1921, lvi, 336.
(35) Starling: Journ. Physiol., 1894, xvi, 224.
(36) Strauss and Chajes: Zeitschr. f. klin. Med., 1904, lii, 536.
(37) Worm-Müller: Ber. d. sächs. Gesellsch. d. Wissensch., 1873, 572.

SUPRARENAL ENLARGEMENT UNDER HEAVY DOSAGE WITH INSULIN[1]

OSCAR RIDDLE, HANNAH ELIZABETH HONEYWELL AND WALTER S. FISHER

From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Received for publication February 6, 1924

In an earlier study Riddle (1) found that pigeons show a high resistance to the lethal action of insulin, these birds often or always surviving the uncomplicated administration of thirty times the proportional amount necessary to kill a one kilogram rabbit. The suggestion was made that some effects of heavy insulin dosage may therefore prove to be more accessible to observation in pigeons than in mammals. Incidental to another study Honeywell and Riddle (2) noted some evidence that single heavy, subcutaneous injections of insulin may lead to fluctuations in the blood sugar concentration of pigeons during as much as a 74-hour period; this concentration after 21 hours in ring doves, perhaps largely according to the dosage used, was sometimes either much below (25 mgm.) or much above (240 mgm.) the normal (149 mgm.). Still other irregularities seemed to attend the long-continued injection of normal birds with a smaller though relatively heavy dosage.

An inspection of the suprarenal weights obtained from a few birds which died after receiving single heavy injections of insulin, and of a few other birds killed for examination soon after the termination of a series of lighter injections, at once suggested that these weights were abnormally high in nearly all cases. On the other hand it was noted that those birds which had last received insulin more than 24 days before their suprarenal weights were taken, or which received the minimum dosage over the shortest period, did not have enlarged suprarenals. Most of the birds used in the earlier studies were not killed for examination soon after being injected; but when the suprarenal data thus obtained were examined they so strongly suggested that the high suprarenal weights were in some way the result of the insulin injections that further tests of the matter were undertaken. Two additional test series have supplied adequate evidence that the suprarenals of pigeons often or usually enlarge after heavy insulin dosage. A few additional tests of the behavior of the blood sugar under these conditions have also been made.

[1] Iletin (Insulin, Lilly) was supplied by the Eli Lilly Co., Indianapolis.

The enlargement of the suprarenals of normal animals under heavy or prolonged insulin injections thus becomes an item of interest in the moot question of the interrelationship of the suprarenals and the pancreas, and of the relationship of the former to the maintenance of the sugar level. Perhaps the results also supply a hitherto unrecognized reason for the avoidance of unusually heavy insulin dosage in man.

Our own special purpose in making this test of the capacity of repeated injections to bring about suprarenal enlargement was, however, as follows: It had been found by one of us (Riddle, data to be published elsewhere) that when such injections were so restricted as to permit ovulation, the size of the ova thus ovulated was measurably reduced. This same change results when thyroid products, or other agents which increase the basal metabolism, are administered to birds. It was therefore necessary to inquire whether the reduced size of ova under insulin is really effected through an increased metabolism due to increased suprarenal action. There is of course some evidence that adrenalin increases the metabolism and that insulin does not. In the present paper, however, the only aspect of this problem which need be considered is the question of increased adrenin production under this type of insulin administration.

CONSIDERATION OF DATA. *Suprarenal weight after insulin.* Three series of observations are included in this report. As noted above, series I includes those birds used in an earlier study (on resistance of pigeons to insulin) which were killed within 24 days after heavy insulin injections. These data (table 1) show that the largest suprarenals were found in those birds which were either given heaviest dosage, or which were killed soonest after the discontinuance of repeated dosage. It is also a suggestive fact that the three birds which died from effects of the heaviest single injections (30 to 33 × "lethal") died sooner or later in the order of their suprarenal weights as found at necropsy. Thus the bird (no. 12) with suprarenals most enlarged died only $8\frac{1}{2}$ hours after injection; the bird with next largest suprarenals (no. 1) died after 21 hours; and the bird (no. 3) with somewhat less enlargement died after 31 hours. Again, of the three birds (nos. 4, 8, 9) given a dosage "10 × lethal" it is the one with largest suprarenals which died; the other two, however, were examined only after 24 days and their glands thus had opportunity to return to normal size. Finally, three birds were given one-sixth unit ("1 × lethal") twice daily during 18, 9 and 3 days respectively; their suprarenal size in each case corresponds to the duration of the dosage. All this may of course be mere coincidence; but we shall see that similar associations reappear in a second and larger group of data.

Parts of the earlier data, as described above, had indicated that a dosage "1 × lethal" (one-sixth unit for birds of 165 grams) given twice daily during 4 to 18 days is sufficient to cause the suprarenals to increase in

weight. In making a second test it was decided to use a dosage three times this strength and to inject it (if the birds would withstand it and continue to take food) three times daily during 7 or 8 days. This dosage was thus given to 15 ring doves and the weights of their suprarenals obtained immediately after the end of the period of treatment. All birds were

TABLE 1

Details of time and amounts of insulin administered to birds of series I. Also time of obtaining suprarenal weights after the last injection. All birds listed in order of suprarenal size—thus showing that this size usually corresponds to the degree of dosage, or to proximity to the last injection

CASE NUMBER	DATA ON INSULIN INJECTIONS			WEIGHT OF:		NUMBER OF ASCARIDIA
	Dosage (Number × lethal)	TIME		Suprarenals	Body	
		Duration (days of of dosage)	Since last dosage			
			Ring doves			
				mgm.	*grams*	
1	33	Single injection	21 hours*	23.6	151	0
2	1	18 (twice daily)	0	21.2	158	3
3	30	Single injection	31 hours*	21.1	132	6
4	10	Single injection	40 hours*	18.6	120	0
5	1.2	4 (twice daily)	0	17.9	133	0
6	1	9 (twice daily)	0	16.0	160	3
7	1	46 (once daily)	0	13.3	176	2
8	10	Single injection	24 days	12.0	177	0
9	10	Single injection	24 days	11.5	173	0
10	1	3 (twice daily)	0	10.8	165	1
11	30	Single injection	35 days	9.8	168	5
Average				16.0	156	
Average (omitting cases no. 8, 9, 11)				17.8	150	
			Common pigeon			
12	33	Single injection	8½ hours*	67.2	317	4

* Died as a result of injection or of this complicated by the drawing of blood samples from the heart; all other birds killed in fair or normal condition. For ring doves, "30 × lethal" means about 5 units of insulin; for common pigeons this amount corresponds to 10 units of insulin.

notably affected by the *first* injection and were dosed twice only on the first day. No injections were made at intervals shorter than 6 hours.

The results of this second test are given in table 2. At least 12 of the birds were found at necropsy to have suprarenals larger than normal. One bird (no. 3) died after only three days of these injections; it then had, with two exceptions, larger suprarenals than the other 14 birds injected

during the full 8-day period. Another (no. 4) died on the eighth or last day of injection and had suprarenals fourth from largest of the group. One bird (no. 7) was killed not at the close of the injection period but 7 days later. All birds were otherwise regularly injected thrice daily until the end of the third day (8/25) when only nos. 9, 12, 13, 14 and 15 were sufficiently active and normal to warrant this injection. These more nearly normal birds, almost without exception, are those which later proved to have the smallest suprarenals of the group. On the following day (8/26) it was necessary to omit the injection of nos. 1 and 2 once and twice respectively; and precisely these two birds had the largest suprarenals of the entire group. These same two birds, together with no. 12, again had to be spared one of the three daily injections two days later (8/28). The average suprarenal size found for the group was 15.0 mgm. The 5 control birds—brothers and sisters of the 15 injected—averaged 10.4 mgm. A second group of 12 control birds (also brothers and sisters) gave an average weight of 11.4 mgm. The question of adequate control must, however, be further considered below.

In connection with this series of measurements a test was made of the possibility that part of the suprarenal enlargement appears within a few hours following insulin administration and disappears within seven days or less. For this purpose eight ring doves (nos. 16 to 23 of table 2) were each given a first injection of about one unit (6 × lethal), followed 24 hours later by a somewhat heavier dosage (10 × lethal). The suprarenal weights of two birds (nos. 16 and 17) were obtained 7 days after injection; for three birds (nos. 18 to 20) these weights were obtained 24 hours after (second) injection; and for a final group of three birds (nos. 21 to 23) only 5 hours after injection. The average for the three groups (table 2) are 13.5, 15.1 and 12.3 mgm. Unfortunately only three of the eight birds used were free from disease (*Ascaridia*) and the data obtained are of uncertain value. The result for birds nos. 21 to 23 would seem to indicate that, under the dosage used, no notable enlargement of the suprarenals occurred within 5 or 6 hours. Other data for other dosage (table 1) indicate that enlargement measurable by weighing did occur within 8½ to 40 hours. The results for nos. 16 and 17 suggest that the enlargement does not persist, at least not completely, during as long as 7 days after injection; after more prolonged dosage (no. 7, table 2), however, there is one item of better evidence for at least a partial persistence. Nos. 18 to 20 do not reliably indicate the degree of enlargement attained at 24 hours.

It was necessary to make the tests of series III late in December. Also, only birds of mature age (6 to 8 months) which had not yet begun to reproduce were available for this study. Since neither suprarenal size nor blood sugar had been studied in such birds at this season it seemed obligatory to run both control and experimental tests and to use only brothers and sisters (two different families) for this work. These precautions proved necessary since neither suprarenal size nor the blood sugar values obtained correspond closely with those found at other seasons of the year. The data from series III therefore can not be directly compared with the

TABLE 2

Suprarenal weights (series II) of 15 ring doves injected with one-half unit insulin ("3 × lethal") one to three times daily during eight days (beginning 8/23). Also, suprarenals of 8 doves injected once (or twice) only with 1.7 units ("10 × lethal") and suprarenals weighed after various intervals. Birds (1–15) recorded in order of their suprarenal size. Blood sugars after 3 and 5 days of repeated heavy injections (one-half unit)

CASE NUMBER	BODY WEIGHT		NUMBER OF ASCARIDIA	WEIGHT OF SUPRARENALS	BLOOD SUGAR FOUND AFTER INSULIN			
					8/25		8/29	8/28-29
	Before insulin	after insulin			2 hours	6 hours	2½ hours	6 hours
	grams	*grams*		*mgm.*				
1	156	134	0	22.2				285*
2	138	101	1	20.2		60		
3	147	132	0	18.2†		90		
4	152	134	0	17.1‡	30			
5	162	153	0	16.3	75		120	
6	155	136	1	15.8	110			
7	161	159	0	14.8§				240
8	158	145	0	14.3		295¶		310
9	154	143	0	14.1	55			
10	160	165	20	14.0			240	235
11	150	134	0	13.5				175
12	137	115	1	12.6				
13	150	130	0	11.6		60	75	295
14	124	125	0	11.2			65	235
15	152	135	0	9.5				375
Ave. (8♂♂)	153	140		16.0				
Ave. (7♀♀)	147	131		13.9				
Ave. (15)	150	136		15.0	(68)	(105)	(125)	(269)
Doves once injected (1.7 units) and killed after various intervals								
16	154	146	0	12.2				
17	156	152	3	14.8				7 days after injection
18	158	142	0	12.9				
19	166	150	5	17.5				24 hours after injection
20	169	149	4	14.8				
21	158	156	10	12.4				
22	149	144	13	13.1				5–6 hours after injection
23	140	136	0	11.3				
Ave. (8)	156	147		13.6				(Healthy and Ascaridia)
Ave. (3)	151	141		12.1				(Healthy only)

* Seven hours after injection 360 mgm. sugar was found.

† Died at end of third day of injections.

‡ Died on eighth (last) day of injections.

§ Killed 7 days after last injection.

¶ On following day (8/26) only 108 mgm. sugar at 6 hours after injection.

earlier data obtained during spring to autumn. But data from experimental and control birds within this series are wholly comparable.

The results are given in table 3 and show that the group of 10 birds injected twice daily during 6 to 7 days with one-sixth unit (1 × lethal) had larger suprarenals (10.4 mgm.) than the 8 controls (8.8 mgm.). Of these 10 birds 6 were given a much heavier injection (1.7 units) at the beginning of treatment. This group of 6, and the remaining group of 4 which was given only the lighter dosage, both showed suprarenal enlargement. The largest gland from a control bird was smaller than the average for the treated group. Both males and females of the treated group show higher suprarenal weights (11.0 and 10.0 mgm.) than do the male and female control (8.9 and 8.6 mgm., respectively). It is thus clear that also in our final test of the matter the results consistently indicate that repeated dosage of doves with insulin, in the amounts stated, leads to a demonstrable increase of weight in the suprarenals.

In the above test we can definitely say that the suprarenal enlargement was not connected with any failure of the birds to take food. Every bird, treated and control, was carefully inspected each afternoon and birds with unfilled crops were crammed with food. The latter procedure was necessary in only two instances. It is true that the birds lost weight as shown in the table; but this loss was quite the same in the blank-injected control animals as in those given insulin.

At this point we may record three recently made tests of the high resistance of doves to insulin and of the probability that the period at which a dove dies after insulin does not always coincide with the period of lowest blood sugar. In Riddle's (1) earlier study of these points it was suggested that the drawing of blood samples (from the heart) for sugar determinations was perhaps partly responsible for most of the few deaths observed up to that time after single heavy injections. Still other data already cited (2) have indicated that the blood sugar reaches its lowest level at from 1 to 4 hours after injection.

For these further tests two ring doves (not fasted; weight 163 grams) were each injected on October 11 with 20 units (about "120 × lethal") insulin. Between 1 and 7 hours after injection these birds were plainly much affected (diarrhea, stupor, impaired vision); most of these conditions persisted, but after this time no new or additional symptoms were observed. The birds were permitted to continue thus until 28½ hours after injection. At this time it seemed probable that the period of lowest blood sugar, and the stage of greatest primary action of the insulin, had been passed. Each bird was then given 0.5 gram dextrose by mouth and a similar amount after a half-hour interval. Grain was forcibly fed after another hour. Twelve hours later the sugar and grain feeding was repeated. Thereafter the forced grain feeding had to be continued—for another 10 days in the case of one bird and for 35 days in the other. During this entire period it was also necessary to give water by placing a filled cup so as to immerse the bird's beak. The large breast areas involved in the injections became indurated or slightly necrotic; but no blood samples were drawn and both birds survived.

TABLE 3

Suprarenal size (late December) after 6 to 7 days, blood sugars after 5 days, of twice daily injections of insulin. (Third series)

NUMBER AND SEX	BODY WEIGHT		NUMBER OF ASCARIDIA	WEIGHT OF SUPRARENALS	BLOOD SUGAR (MGM. PER 100 CC.)				REMARKS ON INSULIN INJECTIONS
	Start	Close			Control	After insulin			
						½ hours	2 hours	6 hours	
Treated									
				mgm.					
1 ♂	183	163	0	11.6		215	160	285	First injection = 1.7 units; later, = one-sixth unit twice daily (5 days)
2 ♀	162	153	0	7.5			65	205	
3 ♂	172	162	0	11.1			130	300	
4 ♀	154	149	0	12.2		180	75	250	
5 ♂	160	147	0	10.8			150	230	
6 ♀	170	158	0	7.2			70	240	
7 ♂	156	149	0	10.6		225	160	170	One-sixth unit twice daily (5 days)
8 ♀	154	134	0	10.6		200	175	270	
9 ♀	169	155	0	13.5			150	250	
10 ♀	156	141	0	9.2			180	220	
Ave.	165	151		10.4		205	132	242	
Control (blank injected)									
11 ♂	171	153	0	9.2	200		220	250	One-sixth unit; once only (for blood sugar)
12 ♂	172	159	0	7.6	175		200	275	
13 ♂	183	168	0	9.0			150	190	
14 ♂	168	159	0	10.1	200		175	240	
15 ♀	161	146	0	8.0	155		125	220	
16 ♀	165	156	2	9.8	100		240	215	
17 ♂	161	147	0	8.8		135*	255	230	Ditto (2 injections)
18 ♀	170	145	0	7.9		150*	215	235	
Ave.	169	154		8.8	166	143*	186	232	
Other control for blood sugar									
19					170		70		One-sixth unit
20					200		110		
21					185		110		One-half unit
22					170		105		
Ave.					181		99		

* These are figures for a 2-hour period (obtained in a second test made 36 hours after the figures placed in the 2-hour column). All blood samples drawn from wing vein.

A third dove was injected with 20 units insulin (120 × lethal) on December 19. Its leg was broken at the time of injection. It showed only slight stupor up to 9 hours. During this period it was plainly less affected than either of the two birds described above. At 19 to 21 hours it showed great weakness and wing tremors and died at 22 to 23 hours. Its suprarenals weighed 11.4 mgm. This is larger than the largest of its control (series III). The results of these three tests, like the earlier ones they were designed to supplement, indicate that doves usually withstand enormous doses of insulin. It also seems probable that few of the deaths observed by us occurred at the time of lowest blood sugar, or as a result of the direct or primary action of insulin.

Control data for suprarenal weight. Known variations and other possible variations in suprarenal size serve to complicate the question of the normal weight of these glands. Data on normal suprarenal size in doves and pigeons have been obtained almost nowhere else than in this laboratory. McCarrison (3) has shown that inanition results in suprarenal hypertrophy in common pigeons, and we have carefully sought to avoid this complication in all except a small part of the data first obtained (series I). In two cases (nos. 3 and 4) of table 1 there was some degree of inanition, and this may have been partially or wholly responsible for the result. If inanition during so short a period as 24 hours results in suprarenal enlargement it is also probable that a very few birds listed in table 2 were slightly affected. It seems quite improbable that the data of series I and II are materially affected from this source. It was noted above that this possibility is wholly excluded in the data of series III. A slight suprarenal size difference in pigeons is probably associated with sex. The presence of round worms (*Ascaridia*) has been shown by Riddle (4) to be frequently accompanied by increased suprarenal size; and this, we believe, is the chief complication encountered in the present data. This complication is present in both the treated and the control animals. Possibly suprarenal size varies in different races or strains. It is now largely unknown whether suprarenal size in pigeons is affected by season. The data of series III suggest a smaller size in December than that found from spring to autumn. In tuberculosis, in other advanced disease, and at ovulation periods these glands are known to enlarge in pigeons (4); but no birds belonging to any of these groups are included in the present study.

A sufficient number of brothers and sisters of the treated birds, all blank-injected, all healthy, and all killed at the same time with the treated birds, would of course provide an unquestionable control. A group precisely such as this is available for only series III. Control "one" (not blank-injected), listed in table 4, are five brothers and sisters of the injected birds of table 2, killed on precisely the same dates as were the insulin injected birds. These three males and two females had suprarenal weights averaging 10.4 mgm. Control "two" (not blank-injected) are twelve other brothers and sisters of the injected birds of table 2, but killed some weeks

or months earlier or later. Their suprarenals average 11.4 mgm. Control "three" is formed of birds (ring doves and common pigeons) similar to the treated birds of table 1; their suprarenals were weighed one to three months later (June) than the treated birds of table 1 and after 6 to 10 days of blank (NaCl) injections made twice daily. The suprarenal size was apparently unaffected by blank injections; the average for six ring doves being 11.5 mgm., and for three common pigeons, 16.4 mgm. Control "four" is the average suprarenal size (11.7 mgm.) of healthy male doves and pigeons as previously published by one of us (4) and as obtained during various months of the year. The conclusion was therefore drawn that the suprarenals of male pigeons are probably slightly larger than those of females. In the several groups of birds used in this study the sexes are represented in approximately equal numbers. Control "five" is the average (10.9) for 14 healthy ring dove hybrids (some of which are represented among the treated birds) not blank-injected and killed at various periods of the year. The weights of glands for healthy birds and for those infested with *Ascaridia* are separately indicated in table 4 and in figure 1.

Another item is considered in connection with these control weights. Before the data of table 2 were obtained it was thought advisable to obtain some light on the effects of repeated subcutaneous injection of adrenalin on suprarenal size. During $4\frac{1}{2}$ days (June) one common pigeon and four ring doves were each given 35 injections of 0.1 cc. of a 1: 25,000 solution of adrenalin chloride.[2] The suprarenal weights found at the end of this period are summarized in table 4. In three of the four ring doves the glands were apparently slightly enlarged, but in the fourth they were abnormally small. The single common pigeon had suprarenals of quite normal size. The dosage received by this bird was, however, only the half of that received by the ring doves, since it was twice the size of the latter and received an identical amount of adrenalin. It is uncertain whether these injections caused any modification in the suprarenal weight of the injected animals, and the weights obtained should probably be considered normal. These weights have some value as (injected) controls.

Table 4 also permits a ready comparison of the average suprarenal weights obtained from the various insulin treated groups and of the various controls in both ring doves and common pigeons. It will be seen that the highest average for any control group is definitely lower than the lowest average for any treated group. These averages for treated birds are, moreover, certainly somewhat reduced by the inclusion (series I and II) of glands obtained either too soon after or too long after the last injection of insulin.

[2] This dosage was probably too small to be of much effect on suprarenal size. A similar dove later survived an injection 25 times this strength.

TABLE 4

Summary on suprarenal size under insulin and control (except series III)

KIND OF PIGEON	TREATED OR CONTROL	SERIES OR GROUP	NUMBER OF BIRDS	AVERAGE			
				Body weight	Suprarenal weight		
					All	Healthy	Ascaridia
				grams	*mgm.*	*mgm.*	*mgm.*
Ring doves	Insulin treated	First	11	156	16.0	16.7	15.4
		Second A	15	136	15.0	14.8	15.6
		Second B	8	147	13.6	12.1	14.5
	Controls	One	5	153	10.4	10.4	
		Two	12	147	11.4	11.0	11.7
		Three*	6	150	11.5	11.5	
		Four	23 ♂	163	11.7	11.7	
		Five	14 ♂	160	10.9	10.9	
	Adrenalin injected		4	138	11.5	11.5	
Common pigeons	Insulin treated	First	1	317	67.7		67.7
	Control	One	3	317	16.4	16.4	
		Two	23	344	19.9	19.9	
	Adrenalin injected		1	276	18.8	18.8	

* Blank injections of NaCl solution.

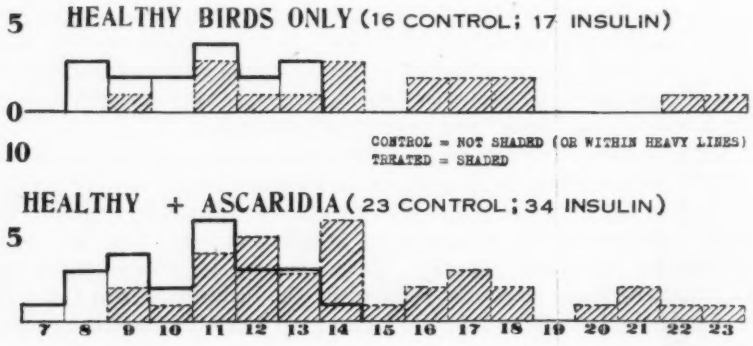


Fig. 1. Histograms of distribution of suprarenal weights obtained from control (groups 1, 2, 3) and insulin injected birds in series I and II. Ordinates represent number of individuals; abscissae represent weight in milligrams.

The distribution of the suprarenal weights of all treated and control ring doves is shown in the histograms of figure 1. The upper part of the figure separately gives the weight distributions in healthy birds only. These histograms, and the details of the various tables, show that the differences found cannot be ascribed to the presence of birds bearing *Ascaridia*. The round worms doubtless affect the weights obtained for both treated and control, but they are not responsible for the differences observed in treated and control.

Blood sugar after dosage with insulin. During the progress of the insulin injections on the birds of series II a few blood sugar determinations were made and these results may next be described. It was not thought advisable to draw blood repeatedly from these birds; but the few data obtained (table 2) show that on the third day of repeated injection the sugar was quite low at 2 hours after injection, and remained low in most cases at 6 hours. This is the same result as was earlier obtained by Honeywell and Riddle (2) after somewhat heavier single injections (10 × lethal). On the fifth and sixth days of repeated injections, however, the sugar was perhaps less reduced at the 2-hour period; and without exception it was *abnormally high* after 6 hours. These blood samples were all drawn from a wing vein.

These data, like the few scattered observations of the earlier study, therefore again indicate that rather heavy and prolonged insulin dosage is followed by marked irregularities in the sugar level. They also suggest that heavy injections of insulin, given repeatedly at short intervals, soon lose their normal capacity to *prolong* a very low sugar concentration in the normal animal. Some of the power seems here to have been lost between the third and the sixth days. Apparently it was lost in the birds whose suprarenals were not abnormally large as well as in those whose suprarenals were enlarged.

These possibilities were again examined in the birds of series III. The results, as shown in table 3, only partially confirm the indications of the earlier data. It is again found that the sugar concentration was extraordinarily high 6 hours after insulin; but this is true not only of the birds which had been subjected to the repeated insulin injections during 5 days, but likewise in the control. Two other confusing results were obtained. First the normal blood sugar of the control birds was notably higher in these birds (December) than extensive observation (5) has shown to be the normal from spring to autumn. These ring doves in other seasons than winter have shown a normal concentration of 149 mgm., but only one of the 9 control birds gave a sugar value approaching this normal figure. One bird, the only one of the series infested with *Ascaridia*, gave an abnormally low value (100 mgm.). If this one value be omitted the other 8 control birds gave an average of 182 mgm. as the normal at this part of the winter season.

The second unexpected result is found in the circumstance that when the control birds were finally injected with insulin, in order to observe the response of the blood sugars to a *first* dose of insulin (exactly equal to that given simultaneously to the repeatedly injected birds), *the sugar at the 2-hour period* (average = 186 mgm.) *was not reduced to the* (December) *normal* in five of the eight cases. In only a single earlier case (table 2) have we failed to find a sub-normal sugar value after two hours, and that case involved a bird which was being subjected to repeated insulin injection. To us it seems probable that this failure to obtain a reduced sugar concentration from the first injection of the control (blank-injected) birds involves some form of proteinic reaction or sensitization.

Such sensitization may conceivably have resulted from the use, during the preceding five days, of the same hypodermic syringe which had each day been used (after rinsing, but not perfectly cleaned) a few moments earlier for the insulin injections. A glance at the 2-hour column of the table will make it entirely evident that the group of blank-injected control did not respond to one-sixth unit insulin as did other control *not* blank-injected, nor as did the 10 birds which during five days had received repeated injections. Moreover, when two of these same birds were given a *second* injection, 36 hours later they showed a lowering (135 to 150 mgm.) at the 2-hour interval practically equal to that of the birds of the repeatedly injected group. In comparing the values obtained in series II with those of series III it should be noted that the dosage used was more than three times larger in the earlier series. Also that the divergence from the normal (149 mgm., August) at 6 hours after dosage, on the fifth day of repeated injection, is greater in the series which was given the heavier dosage.

The data from series III therefore confirm the sugar data of the two earlier series only in indicating that the repeated injection of normal doves with heavy dosage of insulin is usually followed by notable irregularities in the sugar level. The four tested birds of this repeatedly injected series had an average value of 205 mgm. at 14 hours after their eighth injection; their ninth injection was given at the time this blood sample was taken. Two and six hours later these same birds gave values of 143 and 244 respectively. Their normal (December) was probably about 181 mgm. It remains to note that the amount of sugar found at the 2-hour period for the 10 birds of the repeatedly injected group varied greatly—ranging from 65 to 180 mgm. Within this group of birds we are unable to associate lower or higher sugar values with the presence in the same bird of suprarenals of less or greater weight.

Discussion. While this work was in progress a number of studies appeared which bear on the significance and interpretation of the present data. Wilder, Boothby, Barborka, Kitchin and Adams (6), Boothby and Wilder (7), and Cannon, McIver and Bliss (8) obtained results which seem

to indicate that the low sugar concentration following insulin supplies a stimulus to the suprarenals. The last-named workers have made a preliminary report of results which they interpret as demonstrating an increased output of adrenin associated with increased sympathetic discharge, convulsions and coma following insulin.

The suprarenal enlargement reported here seems to provide data of another kind on this response of the suprarenals to insulin or to the low sugar level following insulin. This response by enlargement, the frequent failure of death to occur (where it occurred at all) at the probable time of lowest blood sugar and the individual cases of greater sensitiveness or of injury associated with largest suprarenal size are all appearently capable of interpretation along lines suggested by the studies of the above-mentioned investigators. The heavier glands—probably accompanied by increased function—suggest: First, that the suprarenals take an active part in opposing the effects produced by insulin. Second that the more severe symptoms following heavy insulin in our animals may have been effected in part at least through increased action of the suprarenals; for, in our material we find evidence that the onset of severe symptoms coincides more closely with the probable time of enlargement than with that of lowest blood sugar.

This interpretation is proposed with some hesitation, however, and is acknowledged as subject to limitations in view of the results of three studies by Stewart and Rogoff (9) which have a direct bearing on the present question. Working with adrenalectomized dogs, cats and rabbits Stewart and Rogoff obtained results adverse to the above view. And they conclude from results obtained on animals with intact suprarenals that "insulin does not appear to exert any decided influence upon the rate of output of epinephrin" within 1 or 2 hours and under conditions observed by them. While this *short period* of measurement is not directly concerned in the case of our own data, it apparently is the same period as that covered by the work of Cannon, McIver and Bliss; and it also approximates the period at which convulsions, coma and death appear in most mammals. Sundberg (10) also has reported on the effects of insulin in rabbits deprived of suprarenal medulla. He found that a particular dosage of insulin caused a greater reduction of the blood sugar in the operated rabbits; but, on the other hand, the nervous convulsive symptoms appeared more readily in the operated animals than in the control. The latter observation is difficult to reconcile with the view that increased adrenin secretion, or a suprarenal enlargement, is at all causally related to the sensitiveness or death of these animals after insulin.

In further qualifying our tentative interpretation we would note that the symptoms in birds following heavy insulin can not be said to be very closely similar to those following heavy adrenalin, though diarrhea and

stupor are observable in both. But Wilder has noted that his own subjective symptoms after adrenalin and after insulin were quite similar. And in our own animals it can scarcely be supposed that the effects of excessive adrenalin are unmodified by the presence in great excess of the pancreatic hormone and the other substances included in insulin. We do not wish to imply that only pancreatic and suprarenal hormones are here concerned. Burn (11) has recently shown that pituitrin opposes the capacity of adrenalin to raise the blood sugar and of insulin to lower it; also, that pituitrin effectively prevents the symptoms of hypoglycemia induced by insulin.

A further observation of interest here has recently been reported by Garnier and Schulmann (12). They found that repeated heavy injections of adrenalin in rabbits is frequently followed, after discontinuance of the injections, by a "notable increase in the normal sugar level." If our insulin injections were in fact accompanied by increased adrenin output our animals were really being subjected to "adrenalin dosage" in addition to insulin. And Garnier and Schulmann's results with adrenalin are essentially duplicated by our own findings on the blood sugar after insulin.

The sugar determinations recorded here obtain their full meaning only when they are combined with the data on effects of insulin earlier obtained (series I and several additional birds) by Honeywell and Riddle (2). The chief facts obtained in that study should therefore be summarized here. It was noted that the lowest blood sugars after very heavy insulin (18 to 33 $\times$ lethal) were obtained within 4 hours after injection. Within this period values of 10, 15, 20, 20, 30 and 40 mgm. per 100 cc. were obtained. At 6 hours, values of 40, 50, 85, 90 and 110 were obtained; and at 21 hours the values found were 25, 45, 95, 110, 115 and 235.

Though the above figures indicate lowest blood sugars during the first 4 hours, the death of birds has occurred only after intervals of $8\frac{1}{2}$, 21, 22, 31, 40 and (starvation?) 120 hours. It is probable that the death of two of these birds is partly ascribable to the heart-puncture employed in obtaining these earlier blood samples (2 birds apparently survived a much higher dosage—120 $\times$ lethal). The sugar was determined, however, in only two of the birds that died and in both of these the sugar concentration was probably quite low at death. The common pigeon (no. 12, table 1) which died after $8\frac{1}{2}$ hours showed 20 mgm. sugar at 2 hours and only 10 mgm at 4 hours after injection (normal sugar = 181). The ring dove (no. 3, table 1) which died after 31 hours gave a sugar value 21 hours after injection of only 25—the lowest value obtained after so long an interval. It was nevertheless clear that birds which showed only 20 mgm. sugar 2 hours after injection displayed at that time only slight departures from normal appearance and behavior. We believe the whole of our data suggest that death occurs in these heavily dosed pigeons not at the time of lowest blood sugar but at a subsequent time when the suprarenals have measurably

enlarged. Some of our data indicate that measurable enlargement under heaviest dosage may occur within less than 20 hours.

The macroscopic study of the suprarenals has shown that those obtained after insulin were heavier and larger. The microscopic study of the glands of the dove is attended by considerable difficulty because of the interlaced position of the cortical and medullary elements, and the lack of a uniform distribution throughout the gland. Moreover, in our preparations (bichromate-acetic fixation) we have failed to obtain a satisfactory staining (eosin-hemotoxylin; also Mann's stain) of the nuclei of the medullary cells from either control or injected birds. And only Mann's stain has yielded bright staining granules in the medullary cytoplasm. We do not find it easy to decide whether both cortex and medulla have increased in amount in the glands of the injected birds; but it is clear that the cortical tissue is present in greater relative amount and it is quite possible that the amount of medullary tissue is actually decreased. It also seems clear that the size increase is not due to hyperemia. Our study of the inadequately stained sections of practically all of the glands obtained from the birds of series II, and from a few additional birds, has shown occasional mitosis in the cortex of treated birds and we have failed to find mitosis in the cortex of the control. The medullary nuclei are vesicular and shadowy in both treated and control. We can therefore say nothing concerning hyperplasia in this part of the gland.

When the above facts are associated with the circumstance that short periods of time are involved, and it is remembered that the glands were from birds undergoing slight decrease in body weight, it becomes evident that the size increase was associated with an increased activity of at least the cortical part of the gland. Riddle (4) has earlier shown that enlargement of the gland rapidly and regularly occurs in female doves and pigeons at all ovulation periods; and two of us (13) have earlier shown that the blood sugar increases at these same periods. This proved capacity of the suprarenals of pigeons to undergo rapid enlargement at short recurring intervals in the normal life is probably related to our success in finding enlargement after insulin. The conjunction in the same animal of this readily obtained response, with an ability to survive enormous doses of insulin, has probably specially favored the making of the several observations recorded here.

SUMMARY

The earlier reported fact that normal pigeons survive disproportionately large injections of insulin has been further confirmed and is probably of significance to each of the results described here.

The administration of single heavy doses of insulin, or of repeated less heavy dosage, usually results in suprarenal enlargement which is measurable by weighing.

Some evidence was obtained indicating that repeated heavy insulin dosage is followed within a few days by a lessened capacity of this insulin dosage to maintain the blood sugar at a low level during the usual length of time. Such dosage is also often followed within six hours by abnormally high sugar levels. It seems probable that the time of appearance of the most striking of these irregularities is approximately the time at which enlargement of the suprarenals is demonstrable.

Among the animals studied by us it was mainly those with largest suprarenals which either died or showed most pronounced effects after administration of large amounts of insulin.

To the extent that data obtained on the bird are applicable to the human, these results supply an additional reason for avoidance of heavy insulin dosage in man.

The results indicate that the suprarenals of normal doves make one response which is not merely immediate and quickly transitory to insulin administered heavily or repeatedly. This delayed or prolonged response takes the form of enlargement, certainly involving hyperplasia of the cortex, and is usually associated with an abnormally increased concentration of the sugar of the blood. The data obtained are in harmony with the view that such insulin administration is accompanied by an increased production of adrenin.

BIBLIOGRAPHY

(1) RIDDLE: Proc. Soc. Exper. Biol. and Med., 1923, xx, 244.
(2) HONEYWELL AND RIDDLE: Proc. Soc. Exper. Biol. and Med., 1923, xx, 248.
(3) MCCARRISON: Brit. Med. Journ., 1919, I, 177.
(4) RIDDLE: This Journal, 1923, lxvi, 322.
(5) RIDDLE AND HONEYWELL: This Journal, 1924, lxvii, 317.
(6) WILDER, BOOTHBY, BARBORKA, KITCHIN AND ADAMS: Journ. Metab. Res., 1922, ii, 722.
(7) BOOTHBY AND WILDER: Med. Clinics of North America, 1923, vii, 53.
(8) CANNON, MCIVER AND BLISS: Boston Med. and Surg. Journ., 1923, clxxxix, 141.
(9) STEWART AND ROGOFF: This Journal, 1923, lxv, 319; 331; 342.
(10) SUNDBERG: Compt. rend. soc. biol., 1923, lxxxix, 807.
(11) BURN: Journ. Physiol., 1923, lvii, 318.
(12) GARNIER AND SCHULMANN: Journ. d. Physiol. et Path. generale, 1923, xxi, 92.
(13) RIDDLE AND HONEYWELL: This Journal, 1923, lxvi, 340.

CHEMICAL CHANGES IN THE BLOOD DURING FASTING AND SUBSEQUENT REFEEDING

Experiments on Dogs. I[1]

SERGIUS MORGULIS and A. C. EDWARDS

From the Department of Biochemistry, University of Nebraska College of Medicine

Received for publication February 12, 1924

This investigation is concerned with the changes in various blood components occurring in animals which fast for a long time as well as with the alterations accompanying the recuperation and restoration of the original body weight which follow renewed feeding of the animals. In this first paper we present the results of our study dealing principally with the non-protein nitrogenous constituents of the blood, supplemented however with additional data on the sugar, chlorides and total solids.

Preceding the actual fast each dog was under observation for a certain period during which it was supplied a mixed diet calculated to furnish about 70 calories per kilogram of weight. This diet was found ample to secure a constant weight. The latter condition was regarded as the essential prerequisite before fasting could commence. The animals were also trained in the experimental routine, only those being retained as subjects as had shown themselves thoroughly satisfactory, especially for the bleeding operation.

The bloods were taken directly from the heart, and the dogs were so well trained before the actual experimentation began that they have betrayed no excitement at being fastened to the holder or in the course of the operation. To avoid any possibility of anemic changes supervening, which might become superimposed on the inanition changes in the blood and thus mask or alter the primary effect of fasting, the blood samples never exceeded 15 cc. each, while the bleeding was only performed at definite stages in the progress of the fast. For this reason the first, or preliminary, blood sample was not taken just at the commencement of the fasting but usually two days earlier. While the dogs were fed the blood samples were drawn about twenty-four hours after the last meal (water alone being allowed in the meantime) so that all our analyses are on bloods obtained

[1] The experimental results of this paper are taken from the thesis submitted by the junior author in partial fulfilment of the requirements for the Degree of Master of Arts in Physiological Chemistry.

after the post-absorptive processes have come to an end. Experiments which we performed on blood samples drawn at different intervals following a meal have shown us that an interval of twenty-four hours is entirely reliable, the blood composition being then the same as prior to feeding. The animals' weight was likewise always determined twenty-four hours after feeding, at which time the actual fast began. The weight was measured to 0.01 kilogram.

It is a general practice to follow the progress of a fast, using its duration as an index of the condition of the organism. The fallacy of this procedure has been discussed elsewhere (25). We are using the relative loss in weight during fasting as a basis of comparison and in these experiments the dogs were bled every time the body weight diminished about 10 per cent. During the period of refeeding we were likewise guided not so much by the length of time of refeeding as by the degree to which restoration of the original weight was accomplished.

For the determination of total solids a few drops of the freshly drawn blood (0.1 to 0.3 gram) were placed in a dry, weighed and stoppered test tube. The tube was immediately rotated on its long axis in such a way as to cause the blood to spread in a thin film over the inner surface. The tubes were then reweighed to determine the amount of blood taken. The tubes were then placed in an oven and dried to constant weight at 75°C. This determination was usually done in triplicate.

The bloods were deproteinized according to the method of Folin-Wu. In the tungstate filtrate we determined total non-protein nitrogen, urea nitrogen (by the urease procedure), creatine, creatinine, and sugar by the Folin-Wu system. For the uric acid determination we used Benedict's simplified technic. The amino-acid nitrogen was analyzed by the Folin colorimetric method, and the chlorides according to Whitehorn's procedure.

In the first table we present a summary of the results of our analyses of the dog blood prior to fasting. We supplement our own data with a summary of the data collected from the literature, given in table 2, also pertaining to the composition of dog blood under normal conditions.

The values for the non-protein nitrogen obtained with our dogs are somewhat more variable than those reported by Bang, though the average values are approximately the same. The amounts found by us, however, are lower than those given by Draper. Perhaps the most striking difference is to be observed in the urea nitrogen values as compared to those of Bang, even his lowest figure being higher than our highest results. Although our results are also somewhat lower than those recorded by Draper as well as Atkinson and Ets, they agree very well with the data obtained by a number of investigators (Marshall and Davis, Hammond, Haden and Orr, Van Slyke and collaborators) and we therefore cannot help but feel that Bang's extremely large value may be due to some analytical fault.

The largest series of uric acid determinations in dog's blood was made by Haden and Orr, and the average figure obtained from their analyses of twenty blood samples is practically the same as that which we found for our six dogs.

The studies of György and Zunz on the amino-acid nitrogen in several species of animals show that this component of the non-protein fractions of the blood is very constant. The results of our own analyses made by the new Folin colorimetric method agree neither with those of György and Zunz nor with those of Van Slyke and Meyer. They are, however, in close agreement with the amino-acid values reported by Okada and Hayashi, and by Bock.

Fujii has made a very extensive investigation of the sugar content in dog's blood. The blood sugar values which we find are within the limits

TABLE 1

Normal composition of dog's blood

DOG NUMBER	WEIGHT	RECTAL TEMPERATURE	PER 100 CC. BLOOD								UNDETERMINED N	TOTAL SOLIDS
			Non-protein N	Urea nitrogen	Amino-acid N	Uric acid	Creatinine	Creatine	Sugar	Chlorides		
	kgm.	*°C.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
0	6.68	39.7	32.5	11.1	9.0	0.9	1.6	4.0		352	31	
1	14.18	38.6	32.4	13.8	8.8	0.8	1.5	4.1	74.0	330	23	
2	19.86	38.9	32.3	12.7	7.8	1.3	1.7	2.4	102.0	291	31	
3	12.13	38.8	40.0	15.1	9.5	1.5	1.6	2.4	96.6	334	34	
4	9.58	39.1	40.0	11.1	9.1	2.2	1.5	3.2	104.6	286	44	17.0
5	16.53	39.0	31.9	13.9	7.9	1.1	1.5	2.9	106.3	270	26	19.5

established by Fujii for the normal dog. In the matter of the chlorides there is considerable variability in the data obtained with different dogs but the average value is not sensibly different from the same as given by Hasting, Murray and Murray, or by Haden and Orr.

I. Experiments on fasting with and without water. Experiments on fasting with water allowed *ad libitum* were made on four adult dogs. The weight twenty-four hours after the last meal was taken as the initial weight at the start of the fasting experiment.

During the fast the dogs were weighed regularly, the changes in weight serving as our guide to the progress of the experiment. Samples of blood were taken when a definite change in body weight had occurred. Usually this was done every time the weight had diminished 10 per cent except in the case of dog 1 when the bleeding was done at an approximate loss of 10, 25 and 40 per cent from the initial weight.

As a rule we did not allow our dogs to fast beyond a 40 per cent loss in body weight inasmuch as we were interested in the changes occurring during the refeeding period, and we did not wish to jeopardize their existence by unnecessarily prolonging the experiment. In the case of dog 2, however, the fast was carried until the dog lost over one-half of its

TABLE 2

Composition of normal dog blood

COMPONENT	MGM. PER 100 CC. BLOOD	AUTHOR
Non-protein N	34–38	Bang (4)
Non-protein N	28	Atkinson and Ets (2)
Non-protein N	30.3	Haden and Orr (14)
Non-protein N	44	Draper (7)
Urea nitrogen	17–27	Bang (4)
Urea nitrogen	17.9	Atkinson and Ets (2)
Urea nitrogen	11.1	Haden and Orr (14)
Urea nitrogen	12.5–13	Marshall and Davis (18)
Urea nitrogen	9.2–10.9	Austin, Stillman and Van Slyke (3)
Urea nitrogen	12.1	Hammond (19)
Urea nitrogen	18.0	Draper (7)
Amino-acid N	3–5	Van Slyke and Meyer (33)
Amino-acid N	4–5	Gyorgy and Zunz (13)
Amino-acid N	7.3	Okada and Hayashi (27)
Amino-acid N	7.5	Bock (6)
Uric acid	1.5	Haden and Orr (14)
Uric acid	0.7	Draper (7)
Creatine (young)	1.54	Mathews (19)
Creatine (adult)	2.92	Mathews (19)
Creatinine	1.2–2.0	Haden and Orr (14)
Sugar	80–110	Fujii (12)
Sugar	76	Haden and Orr (14)
Sugar	103	Atkinson and Ets (2)
Sugar	125	Draper (7)
Chlorides	304–306.5	Hastings, Murray and Murray (15)
Chlorides	277	Haden and Orr (14)

initial weight because in this particular instance we felt confident by observing the general condition and the temperature of the animal that we were not running any appreciable risk of losing it unexpectedly. In table 3 we have recorded the results of the analyses of the bloods obtained from the different dogs at different stages in their fast.

TABLE 3

Effect of fasting on the blood

DOG NUMBER	WEIGHT	LOSS	RECTAL TEMPERATURE	PER 100 CC. BLOOD								UNDETERMINED N	SOLIDS
				Non-protein N	Urea nitrogen	Amino-acid N	Uric acid	Creatinine	Creatine	Sugar	Chlorides		
	kgm.	*per cent*	°C.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
1	14.18		38.6	32.4	13.8	8.8	0.8	1.5	4.1	74.0	330	23	
	12.90	9.0	38.6	32.6	13.8	9.1	0.9	1.6	2.3	85.1	302	25	
	10.72	24.40	38.2	32.0	15.0	9.2	1.2	1.7	2.3	60.9	277	20	
	8.65	39.0	36.6	40.5	15.0	8.9	1.5	1.5	3.4	77.2	224	35	19.3
2	19.86		38.9	32.3	12.7	7.8	1.3	1.3	2.4	102.0	291	31	
	17.83	10.23	38.2	42.8	16.1	8.2	1.5	1.6	2.6	76.9	286	38	
	15.95	19.69	38.3	44.1	15.0	9.7	1.4	1.7	2.4	78.8	276	40	17.4
	14.07	29.15	38.2	42.8	16.2	7.7	1.4	1.7	2.4	74.6	226	40	
	12.00	39.58	37.7	42.8	15.4	7.7	1.3	1.7	2.6	83.0	270	41	16.5
	9.95	49.90	38.7	42.2	17.4	7.8	1.1	1.7	2.9	68.2	296	35	17.7
	8.66	56.40	36.4	46.8	21.4	6.2	0.9	1.4	3.4	108.1	320	47	17.3
3	12.13		38.8	40.0	15.1	9.5	1.5	1.6	2.4	96.6	334	34	
	10.66	12.12	39.1	50.0	16.5	9.4	1.9	2.0	2.5	90.9	278	44	17.0
	9.80	19.30	38.1	53.1	17.3	7.7	1.9	1.7	2.8	74.3	296	49	16.9
	8.41	30.67	37.2	49.0	16.8	8.3	1.8	1.6	2.7	89.6	274	45	19.0
	7.32	39.70	37.7	56.0	20.0	10.1	2.1	1.7	3.5	104.7	292	42	20.0
4	9.58		39.1	40.0	11.1	9.1	2.2	1.5	3.2	104.6	286	44	17.0
	8.49	11.38	38.5	44.1	13.5	8.1	2.0	1.6	2.6	84.3	280	46	18.8
	7.66	20.05	38.6	35.6*	15.6	7.6	2.4	1.5	1.8	86.9	264	29	19.0
	6.68	30.90	37.2	43.4	13.8	8.1	2.1	1.7	1.9	85.4	268	45	12.6
	5.78	39.70	36.7	48.3	24.1	9.0	2.7	1.7	2.8	102.5	274	26	13.8
5†	16.33		39.0	31.9	13.9	7.9	1.1	1.6	3.0	106.3	270	26	19.5
	14.64	10.40	38.0	45.8	23.0	7.8	1.4	1.8	2.8	135.1	320	28	20.1
	13.04	20.20	37.6	46.1	17.2	8.8	1.3	1.6	2.3	93.9	298	40	20.7
	11.32	30.70	38.6	54.5	25.0	9.0	1.8	1.9	2.6	100.5	314	34	21.9
	9.82	39.81	37.5	52.6	23.8	8.5	1.9	1.7	3.3	91.3	304	34	23.2

* See footnote 3, p. 491.

† This dog was deprived of food and water.

The foregoing tabulation shows at a glance the general trend of the alterations in the composition of the blood during fasting. For a more definite survey of the results we must consider each component studied separately.

Non-protein nitrogen. Mitchell and collaborators (20) maintain that the total non-protein nitrogen of the tissues is not affected either by the type or intensity of the protein metabolism. This is well substantiated by their experimental results which, however, have only a limited significance because they were obtained from a study of rats which fasted a brief time, twenty-four to twenty-six hours. Since a rat can probably endure a complete fast for several days, the results based on a very short fast are not enough to warrant any definite conclusion with regard to the effect of fasting. As our analyses show, the non-protein nitrogen of the blood increases, especially in the advanced stages of the fast.

At first thought it may seem that the nitrogen retention with the progress of the fast may be due to a condensation of the blood, since the total solids also increase as the fast progresses. The fact that not all the blood components are affected in the same way, nor is the nitrogen retention parallel to the alterations in the total solid residue of the blood would argue against this assumption. Neither can we account for this retention on the basis of kidney involvement inasmuch as the histological picture of the organ even in advanced stages of inanition, as was shown by Morgulis, Howe and Hawk (26) does not reveal sufficient severity to make this supposition probable. Bang (4) noted a marked increase in the "Reststickstoff" (non-protein nitrogen) in a series of fasting rabbits and attributed this to a diminished urinary output of the animals, for if supplied with water the increase during the fasting period was less noticeable. In one of our animals which fasted without water the increase was really somewhat greater than in the series receiving water to drink *ad libitum*.

Considering the changes in the non-protein nitrogen at the different stages of fastings we may come nearer to a true interpretation of the results. The curve of the non-protein nitrogen of the blood follows a course which has been generally observed in inanition (25). The curve rises rather abruptly above the pre-fasting level and reaches a new and higher level at the time when the body weight has suffered a loss of about 10 per cent. This is shown particularly well in our dogs, 2, 3 and 4. In the case of dog 1 we failed to find this initial rise. The non-protein nitrogen level, following the initial rise, remains practically unaltered until the body weight has diminished about 40 to 50 per cent when without exception a second rise occurs and the total nitrogen reaches its highest level. In our dog 2 this second rise did not occur until the loss was 56 per cent of its weight, and we discontinued the fast at that time as there was already too great a danger of losing this animal if food were withheld much longer. The increase in the non-protein nitrogen at this advanced stage of fasting is not regular and represents in dog 1, 25 per cent of the initial level; in dog 2, 48 per cent; in dog 3, 40 per cent; and in dog 4, only 20 per cent. The initial increase in the non-protein nitrogen level of the

blood is probably a physiological change. The fasting animal is suddenly forced to exist entirely on its own tissues. As a result of this the katabolic processes become predominant while the anabolic processes recede in importance and there is, therefore, a new equilibrium established between the destructive and constructive processes in the organism. This determines a new relationship between the tissues and body fluids which manifests itself in an altered nitrogen level in the blood. The second increase which, however, does not happen until a very advanced stage of the fast has been reached, when the bodily reserves are already so greatly depleted that the protein structures are being sacrificed for nourishment, coincides with the fourth inanition period (25). The ultimate great rise in the blood non-protein nitrogen corresponds, therefore, to the premortal increase in the excretion of urinary nitrogen.

Urea nitrogen. The urea nitrogen constituting by far the largest fraction of the total nitrogen, it is not surprising to find that the variations in this component of the blood during fasting follow a course very similar to that of the non-protein nitrogen.

The close similarity between the urea and the non-protein nitrogen curves suggests the possibility that the cause of their variation is the same in both instances, namely, an increased tissue katabolism. Bang (4) thought the increase in the non-protein nitrogen, which he found in the blood of fasting rabbits, was largely due to the urea nitrogen fraction. A comparison of the data dissipates such an assumption, because the increase in total nitrogen is considerably greater than could be accounted for in this way. It is to be remembered that a considerable proportion of the total nitrogen ($\frac{1}{3}$ to possibly $\frac{1}{2}$) is included in what is generally termed as undetermined nitrogen, and this portion tends to increase during fasting. It is more probable, therefore, that the changes in the non-protein nitrogen are associated more directly with alterations in this "undetermined" fraction rather than in the urea nitrogen.

Amino-acid nitrogen. Unlike the urea nitrogen, which as we have just shown undergoes changes definitely related to the changes in total nitrogen and depending upon the stage of the fast, the nitrogen present in the blood in the form of amino-acids is relatively little affected.

Van Slyke and Meyer (34) observed the presence of amino-acids in the blood of fasting dogs and concluded that the amino-acids are intermediate steps not only in the synthesis but also in the breaking down of the body tissues, probably by the process of autolysis. Mitchell and collaborators (20), on the other hand, maintain that the amino-acids of the tissues are not simply intermediate steps in the synthesis and disintegration of protein, but that they perform a distinct and important function in the life activities of the tissues. This accounts for the effective mechanism for keeping their concentration fairly constant. Mitchell thinks that they

may in some way be related to the maintenance of osmotic pressure in the tissues. Dr. C. H. Greene was kind to inform us of some unpublished recent determinations on the amino-acid content in a large number of human subjects, both normal and diseased, which show that the amino-acid content of the blood is practically unchanged except in yellow atrophy of the liver.

In dog 1 the amino-acid nitrogen remained unchanged throughout the fast; in dog 2 there has been likewise little change except for the diminution to 6.2 mgm. (the lowest value found by us in all our analyses) at the time this dog had lost over one-half of its body weight. It will be shown later (table 4) that in dog 1 the constancy of the amino-acid nitrogen level has been maintained also during a repeated fast. Dogs 3 and 4, on the contrary, show a tendency toward a lowering of the amino-acid nitrogen of the blood which lasts until about the middle of the fasting period but increases again thereafter. At the time the fast was discontinued, i.e., when the body weight had suffered a loss of about 40 per cent, the amino-acid nitrogen was once more as high as in the beginning of the experiment. It would seem that the behavior of the amino-acids in the blood is characteristic for each individual.

Marino reports (17) that the amino-acids in the blood begin to increase from the 12th day of fasting and continue to rise until death occurs. Alpern (1) likewise finds a steady rise in the amino-acid fraction in the blood with the progress of the fast in the case of pigeons. The results of both of these investigators are in direct opposition to our findings on fasting dogs.

Uric acid. The uric acid of the blood shows a definite tendency to increase during fasting. In *very* advanced stages of fasting, however, as in the case of dog 2, it may apparently decrease again.

The increase in the uric acid content of the blood, which manifests itself quite early during fasting, may be attributed either to an excessive destruction of nuclear material with a corresponding increase in the amount of purine bases and ultimately of uric acid, or it may be due to a decrease in the formation of allantoin which in the dog is the usual oxidation product of uric acid. The first assumption has little in its favor since it has been shown by Morgulis (21) that the nuclei are quite resistant and do not betray any signs of disintegration except in very advanced stages of inanition. It is still possible, however, that the source of the increased uric acid is derived from leucocytes which it is known do diminish greatly in number during fasting. It is, however, by no means certain that their disappearance from the circulating blood is due to actual destruction. The second possibility seems more likely, namely, that the accumulation of the uric acid may be the result of diminished ability of the organism to form allantoin from the uric acid precursor. The uric acid in

the dog is an intermediary product in the metabolism of the purines and anything which will tend to influence the oxidative function of the tissues will affect the amount of uric acid thus oxidized to allantoin. Pugliese (28) found that the oxidative processes are less intense in fasting than in well fed dogs, and these experiments suggest that the early increase in the blood uric acid may be attributed to the same cause.

It might be argued, of course, that the increase in blood uric acid is due to retention even though there may be no recognizable pathological alteration in the kidneys during fasting. The fact that this accumulation is progressive and commences already at an early stage of the fast deprives the argument of its cogency. Furthermore, the rapid drop in the blood uric acid which occurs when the animals are refed once more would necessitate the assumption that the impaired kidney function is quickly restored to normal as a result of feeding. There is nothing at present to offer in support of such an assumption.

Creatinine. The results of our analyses of the creatinine show very definitely that this component of the blood remains little affected even in the most advanced stages of fasting. We must, therefore, conclude that this substance is evidently independent of the type of metabolism. We are confronted with two possibilities as to its significance: either it is not a metabolic product at all, or it is a product of that stable metabolic process which Folin (8) regards as being kept uniform by a very effective mechanism. The remarkable constancy of the creatinine value under all conditions of fasting forces one to regard it as in some way very essential in the chemistry of the vital process of the tissues.

Creatine. Unlike the creatinine, the creatine of the blood undergoes definite variations during the fast. It decreases at the beginning of fasting and at about the middle of the fasting period reaches the lowest concentration. From that time on, however, it tends to increase once more so that in the very advanced stage its amount may actually exceed that found in the blood at the beginning of the fast. Slosse (31) likewise found that during the years of general undernourishment which prevailed at the time of the World War human blood showed a smaller creatine content. His statement that the creatinine also tended to diminish somewhat is not borne out by our observation on the fasting animals.

Dog 5 fasted without water. The withholding of the water hastens the loss of weight, i.e., increases the rate of the katabolic processes. The total solids of the blood, at any rate at the close of the experiment, when the body had already diminished 40 per cent, is greater than we have found in any of our other dogs. But even in this respect the change is not as striking as one would expect, especially since the initial per cent of solids in this dog's blood was higher than in the other subjects. The changes which we have described before as taking place in the non-protein nitrogen,

urea nitrogen, uric acid, and creatine are practically duplicated under these conditions, but the changes are more marked and greater in amplitude. Thus, the non-protein nitrogen by the end of the experiment is 65 per cent greater than at the start, while the largest rise observed in the dogs provided with drinking water did not exceed 48 per cent when a loss of 56 per cent in body weight was caused. On the other hand, the creatinine and amino-acid values were practically as constant as in the animals drinking water, while the sugar and the chlorides behaved likewise in a parallel manner.

Sugar. During the fasting the blood sugar level changes in an interesting manner. When the body weight has diminished about 20 per cent the blood sugar level is considerably below that existing before the beginning of the fast. This fall in the sugar content may be preceded by a brief and temporary increase (dog 1) but more often the sugar commences to decrease from the very start of the fast. With further progress of the fast the blood sugar tends to increase again until in the very advanced stages it is nearly as high and in some instances even higher than in the normal condition.

The decrease in the blood sugar during the early stages of fasting is probably associated with the exhaustion of carbohydrates from the reserves in the tissues. These, as is well known, are rapidly used up in inanition. The significant fact, however, is the persistence of sugar in the blood long after the tissue reserves are used up and no new supply is furnished by food. It means that the sugar must be continually synthesized from other sources in the fasting organism to maintain a constant supply of this essential material in the cells of the body. The recent investigations with insulin have demonstrated that a certain level of sugar in the blood is a necessary condition for the proper physiological reactions of the organism, hypoglycemia of a certain degree resulting in more or less violent convulsive responses of the animal.

It is obvious that the sugar synthesized in the fasting animal can be derived only from the fat or the protein of the tissues. It is by no means yet clear how this transformation may be accomplished and what the intermediate steps in this metabolism are. It is significant, however, that the rise in the blood sugar level which has been observed to take place during the second half of the fast is synchronous with the increased amount of non-protein nitrogen which also appears in the blood in the advanced stage of fasting. This fact would suggest as a strong probability the formation of the extra sugar from the excessive protein katabolism attending the advanced stage of fasting.

The relative hyperglycemia of the most advanced stage of inanition merits further consideration. The question naturally arises as to the cause for this increased sugar level in the blood, for no matter from what

source it may come, whether from fat or from protein, it is obvious that the hyperglycemia must have its cause in some condition existing in the advanced stage of fasting. It is well to point out that in this stage we are not dealing merely with an hyperglycemia, but also with an increased creatinemia as well as a generally increased non-protein nitrogen level. We cannot attribute the rise in blood sugar level to a sudden flooding of the organism with sugar by-products of metabolism but are forced to conclude that the glycogenetic power of the organism at that stage is seriously impaired. We know that the Islands of Langerhans undergo atrophy in protracted fasting. It has also been shown by Michailesco[2] that while the fasting organism can deposit glycogen during the greater part of the inanition period, this ability is lost in the advanced stages of inanition. It is possible, in view of the knowledge disclosed by recent studies on insulin, that this failure of the organism to accumulate glycogen in the very advanced stages of inanition is associated with a lack of insulin resulting from an atrophy of the island tissue. The hyperglycemia in the last phase of fasting is therefore due to a failure of the liver and possibly of the other organs also to store the extra glucose formed in the metabolism, leading thus to a rise in the blood sugar level. We believe that the conclusion is likewise justified that the creatinemia appearing at this stage is likewise associated with this loss of ability to form and to hold glycogen in the tissues, so that the blood picture in advanced fasting presents a syndrome of metabolic disturbances probably intimately associated with a single cause.

Chlorides. Like the blood sugar, the chlorides decrease during fasting. The lowest level is attained when the fast has progressed about half way. In some cases the decrease continued as long as the fast lasted but more often the chlorides increased once more during the advanced stages. In the case of dog 2 the chlorides at the end of a fast which lasted 87 days even exceeded the original blood chloride level.

It is reasonable to assume that the initial decrease in the blood chlorides is the result of the excretion of excess chloride reserve from the organism. During the early stages of fasting chlorides continue to be eliminated through the urine although the organism receives no new supplies. This naturally leads to a decrease in the amount of chlorides circulating in the blood.

The subsequent rise in the chloride content of the blood is undoubtedly associated with the type of tissue which undergoes destruction to furnish the necessary energy to the fasting organism. It is a well familiar fact, of course, that certain tissues are praticularly rich in chlorides (gastric mucosa, panniculus adiposa) and with progress of the fast these tissues are drawn upon more extensively to provide the metabolic needs of the organ-

[2] Compt. rend. soc. biol., 1914, lxxvi, 314.

ism with the resulting increase in the amount of chlorides. This is especially well shown in dog 2 which has suffered the greatest loss (56 per cent), where with the protraction of the fast there has been a gradual accumulation of chlorides in the blood. It is well known that the stomach and skin, for instance, lose a great deal of their substance when the general loss in body weight has already reached about 30 per cent, and this may explain why the continuous increase in the chlorides has been manifested by this particular dog.

II. Repeated fasting. Two of the dogs which have served as subjects in the first series of experiments were subjected to a second fast after they

TABLE 4

Changes in blood during a second fast

Dog number	Weight	Loss	Rectal temperature	Per 100 cc. blood							Undetermined N	Solids
				Non-protein N	Urea nitrogen	Amino-acid N	Uric acid	Creatinine	Creatine	Sugar	Chlorides		
	kgm.	*per cent*	*°C.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
1	14.18*			32.4	13.8	8.8	0.8	1.5	4.1	74.0	330	23	
	13.85†		39.1	33.7	12.1	7.3	1.0	1.4	2.5	91.3	290	38	14.3
	12.37	10.70	38.5	32.5	13.0	7.1	1.3	1.5	2.7	73.8	280	33	17.7
	11.17	19.40	38.0	32.9	11.9	7.8	1.2	1.6	2.7	73.8	320	35	16.5
	9.79	29.31	37.6	32.2	13.5	8.0	1.1	1.7	2.3	91.7	306	38	
	8.32	39.93	37.7	33.7	11.5	7.9	1.0	1.5	3.0	54.2	354	37	19.9
3	12.13*			40.0	15.1	9.5	1.5	1.6	2.4	96.6	334	34	
	12.34†		38.1	32.7	15.3	7.3	1.2	1.5	2.6	91.7	308	25	14.5
	11.04	10.54	38.0	38.2	14.6	7.1	1.3	1.6	2.4	99.5	270	40	18.1
	9.92‡	19.60	37.9	33.3	13.4	7.8	1.5	1.5	2.6	89.6	304	30	17.3

* Normal composition of the blood before first fast.

† Composition of the blood at the commencement of second fast.

‡ Dog accidentally died.

had regained their normal weight upon refeeding. The effects of the refeeding on the blood composition will be discussed in the next section. Here it will suffice to state that the dogs were given a very liberal diet until they got back to the original weight, when they were again fed in accordance with the general plan pursued in these experiments. The results of this experiment are summed up in table 4.

A comparison of tables 3 and 4 shows that apparently the changes in the blood coincident with a repeated fast are less striking than the changes produced during the first experience. Unfortunately, dog 3 died accidentally before the second fast had progressed very far, but in its first fast

the non-protein nitrogen increased more than 30 per cent at the time it has lost 20 per cent in weight, whereas in the second experiment the non-protein nitrogen values are the same at the beginning of the fast as when a similar 20 per cent loss in weight has been sustained. In the case of dog 1 we observe likewise that the non-protein nitrogen has increased but slightly at the end of the second fast when the animal had lost practically 40 per cent of its weight, whereas in the first fast it had increased about 25 per cent.

As regards the other blood constituents the survey of both tables leaves no doubt that the general trend of the changes are essentially the same in the two consecutive fasts, but the changes are of much smaller amplitude during the repetition of the fast.

A study of these results showing a diminished effect of repeated fasting upon the blood composition suggests that this effect must be due to a more constant rate of metabolism and an increased resistance of the tissues to disintegration. These results on the changes in the blood during repeated fasting present, therefore, from another side the condition already found by Howe (16), namely, that the nitrogen excretion diminishes when the subject is submitted to fasting for the second time.

III. Effect of refeeding on the blood. Each of the five dogs which were used in the experiments for the determination of the blood changes produced during prolonged fasting was refed until the original body weight was practically restored. During this period of refeeding the blood was studied at intervals. The procedure of obtaining the blood samples as well as the analytical methods were the same as those employed in the previous experiments. The conditions under which the animals were kept during this part of the experiment were likewise the same as before, except that they were now fed daily at a definite time and a definite food mixture.

We varied the composition of the food mixture for each dog, but the daily rations were calculated to provide our dogs, at any rate in the beginning of the feeding, two to three times the usual energy requirement. Only on the first day of breaking the fast were the animals given a half ration, otherwise the forced feeding was continued so long as the dogs ate the food willingly.

The first refeed sample of blood was usually obtained after the fifth day of feeding, and subsequent samples, at least for a time, were also taken at five-day intervals. When the animal had regained its former, i.e., pre-fasting, weight a final blood sample was again analyzed.

In table 5 we record the composition of the various daily rations.

The results of the analyses of the blood at various stages in the refeeding period are summarized in table 6. It will be observed that the refeeding was continued for 37 to 57 days in the case of dogs 1 to 4, while in the case of dog 5 the observations were extended until the fourteenth day of feeding.

TABLE 5

DOG	DIETARY ARTICLE				APPROXIMATE COMPOSITION			CALORIES
	Meat	Rice	Bread	Lard	Protein	Fat	Carbohydrates	
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	
1	250	125		25	54	65	99	1190
2	350		150	40	79	99	107	1565
3	150		200	20	50	48	142	1200
4	200			50	35	82		883
5	300		150	25	89	76	107	1570

TABLE 6

Effect of refeeding on the blood

DOG NUMBER	WEIGHT	GAIN IN WEIGHT	DIFFERENCE BETWEEN THIS AND INITIAL WEIGHT	DURATION OF REFEEDING IN DAYS	MGM. PER 100 CC. BLOOD								UNDETERMINED	SOLIDS
					Non-protein N	Urea nitrogen	Amino-acid N	Uric acid	Creatinine	Creatine	Sugar	Chlorides		
	kgm.	*per cent*	*per cent*		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
1	8.65		39.0	0	40.5	15.0	8.9	1.5	1.5	3.4	77.2	224	35	19.3
	9.58	10.8	32.4	5	31.4	7.5	8.9	1.1	1.5	2.9	76.0	324	42	12.6
	10.52	21.6	25.8	10	29.2	9.0	8.0	0.8	1.6	2.6	96.0	268	36	10.4
	11.66	34.6	18.5	15	26.5	10.0	7.3	0.8	1.7	1.8	93.0	300	29	10.0
	13.85	60.1	9.4	37	33.7	12.1	7.3	1.0	1.4	2.5	91.3	290	38	14.3
2	8.66		56.4	0	46.8	21.4	6.2	0.9	1.4	3.4	108.1	320	37	17.3
	10.47	20.9	47.3	5	46.1	19.3	7.1	0.8	1.4	2.6	90.9	330	39	12.7
	11.19	29.2	43.6	10	29.2	7.4	6.3	0.9	1.3	2.7	94.3	270	48	14.3
	12.00	38.5	38.1	15	25.9	11.4	7.8	0.8	1.5	3.2	111.1	316	19	12.3
	13.49	55.8	32.0	26	30.6	10.0	8.2	0.9	1.3	2.5	90.9	294	35	16.1
	21.70	150.8	+9.2	57	31.5	12.9	8.7	0.9	1.4	2.6	96.1	275	26	16.0
3	7.32		39.7	0	56.0	20.0	10.1	2.1	1.7	3.5	104.7	292	42	20.0
	9.15	25.0	24.6	5	25.0	12.5	9.9	1.3	1.4	2.4	115.0	280	5	10.6
	10.19	39.3	16.4	10	29.4	11.4	8.1	1.3	1.5	2.3	111.7	288	28	13.7
	11.44	56.3	6.2	16	29.7	9.0	7.7	1.3	1.7	1.8	105.2	276	38	11.6
	12.34	68.6	+1.7	37	32.7	15.3	7.3	1.2	1.5	2.6	91.7	308	25	14.5
4	5.78		39.7	0	48.3	24.1	9.0	2.7	1.7	2.8	102.5	274	26	13.8
	6.19	7.1	35.4	4	34.8	16.6	7.8	2.0	1.6	2.5	100.5	270	29	15.3
	6.59	14.0	31.2	19	33.3	13.9	7.7	1.8	1.5	2.5	87.3	300	39	13.4
	7.28	26.0	24.1	14	40.5	17.4	9.3	2.0	1.4	3.3	112.0	290	30	15.7
	9.26	60.2	5.4	46	33.5	13.2	8.1	1.5	1.5	2.5	103.6	306	30	17.0
5	9.83		39.8	0	52.6	23.8	8.5	1.9	1.7	3.3	91.3	304	34	23.2
	12.77	30.1	21.8	7	26.0	10.8	7.2	1.0	1.4	2.7	94.0	278	24	19.0
	14.28	45.3	12.7	12	26.3	7.6	8.0	1.0	1.5	2.5	90.5	308	34	17.1

Two of the refed dogs had not merely regained their original weight but were in fact somewhat heavier (1.7 and 9.2 per cent respectively) at the time the last refeed blood sample was taken. Dogs 1 and 4 were still somewhat under the original weight (5.4 and 9.4 per cent respectively) while dog 5 which was fed only a fortnight was still 12.7 per cent below its weight at the commencement of fasting at the time the second refeed sample of blood was analyzed.

Surveying the data presented in the foregoing table we note certain changes in the blood composition which occur in all animals and which we may, therefore, regard as characteristic and significant for the metabolic phenomena accompanying this period of recuperation from prolonged fasting.

Non-protein nitrogen. Our analyses show that after a few meals the non-protein nitrogen of the blood begins to decrease, this decrease persisting until the animals have gained 35 to 45 per cent in weight.[3] It will be recalled that toward the end of the fast the non-protein nitrogen had increased considerably, but during the refeeding the total nitrogen falls below the level originally found in the dog's blood. When the processes of restoration have progressed so far that the animal's weight is already about 40 per cent greater than at the close of the fast the non-protein nitrogen of the blood begins to increase once more. By the time the original body weight had been practically regained the non-protein nitrogen in the blood of dogs 1 and 2 is the same as at the beginning of the fast. In the case of dogs 3 and 4 this is still somewhat lower than at the beginning, but it will be noted that in these two dogs the non-protein nitrogen was rather high before they began fasting. In dog 5 the refeeding had not advanced far enough to restore the original condition of the blood though the initial changes are typical in every essential.

[3] In this connection it is interesting to record an experience with dog 4 which goes to show that the metabolic reaction resulting from feeding following a preliminary fast is more far-reaching than is commonly suspected. When this dog 4 had already lost 18 to 19 per cent in weight it accidentally gained access to food. Although this was immediately discovered it was impossible to determine the amount of food which the dog actually consumed. Since its body weight soon after eating was found to be only about 15 per cent below its initial weight, it is reasonable to assume that the quantity of food probably did not exceed 5 per cent of the dog's weight, i.e., about 400 grams. The dog was again isolated, and we have had no other accidents of this nature in the course of these experiments. When the dog fasted long enough to diminish 20 per cent in weight a sample of blood was taken as usual for analysis. This, of course, was done several days after the accidental feeding occurred. The non-protein nitrogen of the blood, however, showed a very marked diminution at that time (see table 3). We were unable to explain this great change in the non-protein nitrogen but satisfied ourselves by repeated analyses of the same blood that this was not due to an analytical error. It was only later, when we gained systematic knowledge of the effect of refeeding on the different blood components, that this accidental result became clear to us.

It is well to note that apparently these changes occasioned in the non-protein nitrogen of the blood are independent of the protein content of their diet, as the same general changes were found while the approximate protein content of the diets varied from 35 to 89 grams per day.

Bang (4) also observed this initial decrease in non-protein nitrogen of the blood following a fast. It is perhaps worth mentioning in this connection that Sedgwick and Ziegler (29) found that in infants three to forty-three days after birth the non-protein nitrogen of the blood shows a similar trend, namely, to decrease somewhat rapidly from over 50 mgm. on the third day of age to about 30 to 35 mgm. at the age of ten days, after which time the change in the non-protein nitrogen of the blood becomes less uniform or extensive. It is a familiar fact that the new-born infant loses weight during the first few days of its extra-uterine existence and it is probable, in the light of the results of our investigation, that the high non-protein nitrogen level in infants under five days of age is likewise an inanition effect, the subsequent rather abrupt diminution with improved nourishment further substantiating this hypothesis.

The non-protein nitrogen level of the blood is regulated, on the one hand, by the rate of nitrogen elimination, and, on the other, by the exchange between the tissues and blood. One may expect, therefore, that the sudden abrupt diminution of the nitrogen of the blood following refeeding would result either from an increased elimination or from an increased withdrawal of nitrogenous material by the tissues. Howe (16) has actually observed that during refeeding following a fast the nitrogen elimination in the urine may be increased. Nevertheless, it seems highly improbable that this mechanism is responsible for the diminution of the total non-protein nitrogen of the blood, first, because the increased urinary nitrogen output found by Howe is hardly sufficient to constitute such a heavy drain upon the circulating non-protein material in the blood; secondly, because the increase in nitrogen output is of the same order of magnitude as that following a meal in a normal subject where no alteration of the blood nitrogen level is known similar to the decrease which our refed starved dogs reveal during a considerable period of renewed abundant feeding.

It seems much more probable that the lowering of the nitrogen level is in some manner associated with an altered balance between the body tissues and the blood. We may regard this change as being due to a greater withdrawal of the non-protein constituents from the blood into the tissues where they are utilized in the anabolic processes of tissue regeneration.

Urea nitrogen. The urea nitrogen of the blood likewise diminishes during the period of refeeding. The general course of the changes in urea nitrogen is practically the same as in the case of the non-protein nitrogen, but the diminution is much greater than in the latter, the urea

nitrogen decreasing to about $\frac{1}{3}$ to $\frac{1}{2}$ of the value obtained at the close of the fast. As in the matter of the non-protein nitrogen, however, so also the urea level after reaching a minimum tends again to return to the pre-fasting condition. In the cases of the infants, studied by Sedgwick and Ziegler (29), a similar situation appears, the urea nitrogen, at least in two of the infants, diminishing to approximately $\frac{1}{2}$ the amount found in the earlier stages. This similarity between the changes in non-protein nitrogen and urea nitrogen in infants during their first ten days of extra-uterine existence and of the refed fasting dogs suggests that in the infants we are likewise dealing with post-inanition changes.

The cause of the diminished urea nitrogen level in the blood is probably a reduced formation of urea in the metabolism of protein material by deamination of the amino-acids, the amino-acids being largely utilized in the synthesis of protein of the regenerating tissues. As a result of this deviation of the amino-acids from the usual metabolic course the urea nitrogen of the blood undergoes an extreme diminution in concentration during feeding which follows a long fast.

Amino-acid nitrogen. Considering the great changes which have been described in the non-protein and urea blood nitrogen, the variations in the amino-acid content of the blood during the period of refeeding are certainly slight. The changes are not uniform, though a tendency to a small initial reduction of the amino-acid level is evident. In three of the refed dogs this diminution is followed by an increase in the later stages of refeeding, while in two dogs the diminution though small continues all through the refeeding period during which the animals were under examination. The amino-acid nitrogen content at the close of the period is almost the same as before the dogs commenced to fast.

During the period of refeeding after a previous long fast, as in the course of the fast itself, the amino-acids are one of the least variable components of the non-protein nitrogen of the blood which gives support to the conception that they must be necessary for the life of the animal and that there must, therefore, exist an efficient mechanism for regulating the equilibrium which prevails between the free amino-acids of the tissues and of the blood.

Uric acid. We have already found out from the foregoing discussion that as a result of metabolic alterations occurring under the conditions of fasting the uric acid in the blood tends to accumulate. The accumulation is a progressive phenomenon. Under the influence of refeeding the opposite phenomenon, namely, the rapid reduction in the uric acid concentration in the blood becomes at once evident. Dog 2, which fasted until it sustained a loss of 56 per cent in weight, showed a slight decline in the uric acid concentration in the advanced phases of its inanition. The uric acid of the blood in this dog diminished below the normal level when feeding

was resumed. The progressive rise in uric acid during fasting was attributed to the reduced metabolic rate and diminished oxidation. During refeeding, however, metabolic activity is raised to a higher plane and the processes of oxidation in the body become intensified, as was shown by Pugliese (28) and Morgulis (25). This evidently leads to an increased formation of allantoin and a consequent lowering of the uric acid content of the blood.

Sedgwick and Kingbury's (30) studies on the blood uric acid of newborn infants offers a very striking analogy to the results of our experiments with the refed starved dogs. The uric acid content of the blood of these infants increased from 3.0 to 3.9 mgm. per 100 cc. blood from the time of birth until the end of the third day of extra-uterine existence. It is well to remember that this is usually a period of inanition of the new-born. But following this the uric acid content of the blood rapidly falls and at the age of eight to eleven days reaches the normal value for human blood of about 2 mgm. In other words, the increase in uric acid which was an inanition phenomenon is replaced by a decrease as soon as the infant commences to feed. In the light of our inanition experiments on dogs these blood studies on the infants gain significance.

Creatinine. The creatinine content of the blood is least affected during the period of refeeding.

Creatine. The creatine, on the contrary, tends to decrease during the refeeding. In the advanced stages of inanition, it has been shown before, this blood constituent increases considerably in amount but as soon as feeding is resumed it diminishes. The tendency to regain its original level as the body weight is restored to the pre-fasting condition is revealed also by the blood creatine. We believe that Sedgwick and Ziegler's results on the blood of new-born infants with regard to the creatine show the same relationship though the results are not as uniform as was the case with the other blood components discussed before.

In the preceding chapters it was suggested that the creatine content of the blood varies with the intensity of the protein metabolism. During the period of refeeding the decrease in the creatine of the blood, which may even fall below the normal level at certain stages, coincides apparently with the extensive anabolic processes of muscle regeneration.

Sugar. With the beginning of re-alimentation the behavior of the blood sugar is different in each dog. In dog 1 we find that for the first five days of refeeding the sugar level remains practically the same at the end of the inanition experiment, which is also true for dog 4, while dog 5 shows no change of any significance in the sugar level even at the end of twelve days of feeding. Only in the case of dog 2, in which there was a very striking premortal rise in the blood sugar (from 68 to 108 mgm. at the loss of 49.9 and 56.4 per cent in body weight respectively), did the blood sugar drop

to a lower level within the first few days of refeeding, retaining this level practically unchanged until the dog's weight was very nearly restored to the pre-fasting condition. In the case of dog 3 there was an actual rise in the blood sugar level which probably commenced with the refeeding. The high sugar level persisted for a considerable length of time, though following the initial rise the level again began to fall slowly and after 37 days of feeding, when the original body weight of the dog was practically restored, the blood sugar level was again as under the normal condition.

The increase in the blood sugar in dog 3 would seem to indicate that this particular animal's power of carbohydrate utilization has suffered as a result of inanition. When we examine, however, the dietary records given in table 5 it appears that this dog was given a diet particularly rich in starchy material (bread), the carbohydrate moiety of this mixture supplying considerably more than one-half of the total calories. The rise in sugar level persisting even twenty-four hours after feeding is, therefore, the result of flooding the body with carbohydrate. Since no analysis of the urine was made it is not possible to say whether the increased sugar level was associated with a glycosuria.

Not all of the sugar of the blood is, however, of exogenous origin. The case of dog 4 gives interesting information on this point. By referring to table 5 it will be noted that the dietary of this dog was in some respects the exact opposite of that used for dog 3. The diet of dog 4 consisted of meat and lard only; it contained, therefore, only negligible amounts of free carbohydrate. This diet was low in protein but extremely high in fat, about $\frac{8}{9}$ of the total energy supplied being in the form of lard. It will be noticed, however, that the blood sugar level of this dog remained high and practically at the same height as in its pre-fasting condition. It is obvious that in this animal the sugar in the system must have been of endogenous origin. It is also impossible to avoid the conclusion that the hyperglycemic condition of dog 3 was due to the fact that the diet was predominantly carbohydrate. This hyperglycemia was only temporary and when examined after thirty-seven days of refeeding the blood sugar was already essentially normal.

The undetermined portion of the total non-protein nitrogen which we calculated by subtracting the nitrogen value of urea, amino-acid, uric acid, creatine and creatinine from the total nitrogen value and regarding the latter as one hundred per cent, shows a tendency to increase during fasting which appears regularly in four out of our five animals. In dog 4, although the changes in the undetermined nitrogen of the blood are not contrary to what we have found in the case of the other dogs, there is no actual increase and toward the end of the inanition period the value falls below the normal. The behavior of the blood of this particular dog was atypical in some other respects also, as will be mentioned presently. This tendency

for the increase in the undetermined nitrogen is seen clearly in the repeated fasts, and especially in the dog which fasted without water. During the refeeding, owing to the rapid diminution of the non-protein nitrogen, the undetermined nitrogen fraction usually falls very markedly, though in the case of dogs 1 and 2 a rise in the per cent of undetermined nitrogen preceded this fall. Ultimately, in our experimental animals we found that the undetermined nitrogen fraction of the blood is restored to a value very nearly the same as that found in the pre-fasting period. The degree of the diminution of the undetermined nitrogen fraction depends entirely on the rapidity with which the very high total nitrogen level attained at the close of the fast is reduced. In dogs 4 and 5 this diminution is definite but not as striking as it is in the dog 3 where immediately upon re-alimentation the undetermined nitrogen diminishes to only 5 per cent of the total, the lowest value ever found by us. The great réduction of the total nitrogen of the blood and its concomitant, the fall of the per cent of the undetermined nitrogen, leads us to believe that, as was already suggested previously, in the advanced stages of fasting end products of protein metabolism appear in large amount different from the nitrogenous substances which are accessible to our analytical procedure. The condition existing at the end of protracted fasting is apparently not similar to the nitrogenous retention observed in nephritis where owing to the great increase in the urea nitrogen fraction of the blood, the undetermined nitrogen of the blood remains somewhere about 10 to 15 per cent of the total non-protein nitrogen.

Unfortunately, we did not begin to make determinations of the total solids until several inanition experiments were already under way for some time. The data are, therefore, fragmentary for dogs 1 and 2, while for dog 3 the normal value is missing. The data, however, are complete for two dogs, 4 and 5, and also during the repeated fasts as well as during the re-alimentation following fasting.

In dog 1 during the repetition of the fast the solids increase gradually until at the time of 40 per cent loss in weight it reaches 19.9 per cent, which is very nearly the per cent we found in the same subject at the end of the first fast, namely, 19.3 per cent (the per cent of solids is calculated on the basis of weight). Dog 3 showed the same gradual rise in the total solids during both its fasting experiments, and the same was found even more strikingly in dog 5 which fasted without water. Dog 4 behaves atypically also in respect to the total solids in the blood, these increasing from 17 to 19 per cent up to the time of a 20 per cent loss in weight. During the subsequent part of the fast, while its weight diminished about 40 per cent, the per cent of solids dropped off sharply. On refeeding we found in four of our dogs that even in the first few days the blood becomes much more fluid, and the per cent of solids diminishes very appreciably,

except in the case of dog 4 where instead a small increase in total solids occurred at the beginning of re-alimentation. Ultimately, however, as the animal's weight is gradually being restored to the pre-fasting state, the per cent of total solids in the blood also reaches the normal level.

SUMMARY

1. The non-protein nitrogen and the urea nitrogen of the blood usually increase at an early stage of fasting and remain at a more or less fixed level until the extreme stage is reached when a new and much greater increase occurs. The amino-acid nitrogen either remains constant all through the duration of the fast or may diminish slightly at first to rise once more during the later part of the fast. The blood uric acid increases progressively during fasting. The creatinine remains constant but the creatine, following a diminution which may occur during an early stage, rises rapidly in the most advanced stage of fasting. The sugar and chlorides which usually diminish in the course of the first half of the fasting period increase again during the later stages. At the time the body weight has lost about 40 per cent or more, the blood sugar level may even exceed the normal. The undetermined fraction of the non-protein blood nitrogen increases in course of fasting as does also the per cent of total solids (one dog behaved contrary to this general rule).

2. Fasting without water seems to exercise no other effect upon the blood except to exaggerate the inanition effects already enumerated.

3. However, on repeating a fast the blood changes may become less pronounced or even fail to appear altogether. The total solids increase as they also did during the first fast.

4. When following a protracted fast, the animals are fed once more very extensive changes occur in their blood. The non-protein and urea nitrogen, the uric acid and creatine decrease rapidly even in the first few days of re-alimentation. The decrease in non-protein nitrogen and in urea nitrogen is very striking. When, however, the refed animals have gained 35 to 45 per cent in weight the urea and non-protein nitrogen begin to increase once more tending to return to the original level by the time the pre-fasting weight is about restored. The amino-acid nitrogen undergoes slight changes while the creatinine is entirely unaffected by the refeeding. With the commencement of re-alimentation the per cent of total solids in the blood falls very abruptly. As the refeeding progresses and the original body weight becomes restored the blood composition becomes again practically normal.

BIBLIOGRAPHY

(1) Alpern: Biochem. Zeitschr., 1923, cxxxviii, 142.
(2) Atkinson and Ets: Journ. Biol. Chem., 1922, lii, 5.
(3) Austin, Stillman and Van Slyke: Journ. Biol. Chem., 1920, xlvi, 91.
(4) Bang: Biochem. Zeitschr., 1915, lxxii, 104.
(5) Benedict: Journ. Biol. Chem., 1922, li, 187.
(6) Bock: Journ. Biol. Chem., 1917, xxix, 191.
(7) Draper: Journ. Amer. Med. Assoc., 1917, lxix, 1768.
(8) Folin: This Journal, 1905, xiii, 117.
(9) Folin: Journ. Biol. Chem., 1922, li, 377.
(10) Folin and Wu: Journ. Biol. Chem., 1919, xxxviii, 81.
(11) Folin and Wu: Journ. Biol. Chem., 1920, xli, 367.
(12) Fujii: Tohoku Journ. Med. Exper., 1922, iii, 74.
(13) György and Zunz: Journ. Biol. Chem., 1915, xxi, 511.
(14) Haden and Orr: Journ. Exper. Med., 1923, xxxvii, 365; xxxviii, 477.
(15) Hastings, Murray and Murray: Journ. Biol. Chem., 1921, xlvi, 223.
(16) Howe: Journ. Amer. Chem. Soc., 1910, xxxiii, 215.
(17) Marino: Arch. farm. sper., 1923, xxxvi, 56.
(18) Marshall and Davis: Journ. Biol. Chem., 1914, xviii, 53.
(19) Mathews: Physiological chemistry. New York, 1920.
(20) Mitchell, Nevens and Kendall: Journ. Biol. Chem. 1922, lii, 417.
(21) Morgulis: Arch. Entwicklungsmech. d. Organ., 1911, xxxii, 169.
(22) Morgulis: Biochem. Bull., 1914, iii, 264.
(23) Morgulis: Journ. Biol. Chem., 1922, l, lii.
(24) Morgulis: Carnegie Institution Year Book no. 21, 1922, 173.
(25) Morgulis: Fasting and Undernutrition. New York, 1923.
(26) Morgulis, Howe and Hawk: Biol. Bull., 1915, xxviii, 397.
(27) Okada and Hayaski: Journ. Biol. Chem., 1922, li, 121.
(28) Pugliese: Arch. ital. biol., 1893, xix, 364.
(29) Sedgwick and Ziegler: Amer. Journ. Dis. Child., 1920, xix, 429.
(30) Sedgwick and Kingsbury: Amer. Journ. Dis. Child., 1917 xiv, 98.
(31) Slosse: Soc. roy. sci. méd. et anat. de Bruxelles, 1922, 155.
(32) Whitehorn: Journ. Biol. Chem., 1921, xlv, 449.
(33) Van Slyke and Meyer: Journ. Biol. Chem., 1912, xii, 403.
(34) Van Slyke and Meyer: Journ. Biol. Chem., 1913, xvi, 231.

STUDIES OF THE BRAIN STEM

VIII. Diuresis and Anhydremia Following Destruction of the Thalamus

FRED T. ROGERS

From the Physiology Laboratory, Baylor Medical College, Dallas

Received for publication February 18, 1924

Several years ago while studying the effects of experimental lesions of the thalamus in the bird, it was observed (1) that in many cases a rapid loss of body weight with an apparent diarrhea followed a gross thalamic lesion. As a result it proved difficult to keep such animals alive for any great length of time. Further analysis showed that an additional factor was involved, and that was the inability of such animals to maintain the normal body temperature. In the bird, decerebration with destruction of the entire thalamus constantly leads to these two effects, a poikilothermous condition and rapid loss of body weight.

It was at first thought that the loss of body weight might be due to altered metabolic changes dependent on the brain lesion. We have however shown recently (2) that the metabolic rate as measured by oxygen consumption and carbon dioxide elimination shows no appreciable change after loss of the thalamus and cerebral hemispheres, if the body temperature is artificially maintained at the normal level. With a constant body temperature no direct relation of thalamic activity to basal metabolism, as measured by oxygen consumption, could be discovered. However as a further check on this point measurements were made of the amount of solids eliminated by the intestinal and urinary tracts. This seemed particularly urgent, inasmuch as Freund and Gräfe (3) have stated that rendering the rabbit poikilothermic by spinal transection markedly increases the nitrogen output.

Since the urinary and intestinal excretions of the bird are mixed in the cloaca before elimination from the body it is difficult to collect either urine or feces without admixture of the one with the other. For the purpose at hand the excretions had to be collected, without manipulation of the bird, over periods of time of four to six days. The following standard procedure was employed. The pigeons either normal or decerebrate were starved for 24 hours. They were then kept in a darkened incubator at 30°C. throughout the determination. These conditions

were chosen because determinations of the metabolic rate showed that at the atmospheric temperature of 30°C. the oxygen consumption is at a minimum (2), and because at this atmospheric temperature the poikilothermic decerebrate pigeon maintains a body temperature very close to the normal values of 40° to 42°C. The mixed urinary and intestinal excreta were collected on clean dry paper, dried at 100°C. for 24 hours and then weighed. The excreta obviously contained the mixed biliary, urinary and intestinal components. Since however the animals were fasting, the dry weight of the intestinal excreta except for the bile compounds is negligible, the total mass is principally uric acid, and this figure serves as an approximate index of the total nitrogen metabolism.

It is important to note that in all the determinations here reported the following standard conditions were maintained: first, no food or water for 24 hours before any experimental procedure or measurement was carried out; and second, maintenance of an atmospheric temperature of 29° to 31°C. and body temperature of 39° to 42°C.

A comparison of the weights of the dry urinary and intestinal excreta of the pigeon in the normal homothermous and decerebrate poikilothermous conditions, is given in table 1. It will be seen from this table that after destruction of the thalamus, there is a slight increase in the amount of urinary and intestinal solids eliminated. Thus the average for the normal homothermous bird is 0.7 gram per 24 hours; for the poikilothermis decerebrate the average value is 1.1 gram per 24 hours.

This increase of 0.4 gram per 24 hours does not account for the great loss of weight exhibited by the poikilothermic bird as compared with the normal bird. After destruction of the thalamus the pigeon under the specified standard conditions, loses weight at the rate of 30 to 60 grams per 24 hours (table 3). Even if it be assumed that the weight of the excreta were that of nitrogen only instead of uric acid, the 0.4 gram would correspond to less than 3 grams of dry protein. The predominant loss of body substance in the above condition is therefore not protein.

Measurements of the respiratory quotient of pigeons under the specified standard conditions gave values of 0.70 to 0.75. With a respiratory quotient of this value the weight of the carbon dioxide excreted is nearly balanced by the weight of the inspired oxygen. Thus in one determination (2) the respiratory quotient was 0.72 with oxygen consumption of 290 cc. per hour and carbon dioxide excretion of 210 cc. per hour. Calculation for the twenty-four hour interval shows that 9.9 grams of oxygen was absorbed and 10.9 grams of carbon dioxide excreted. Under standard conditions therefore the great loss of body weight is not due to excessive elimination of carbon dioxide. These considerations therefore focused attention on the water content of the body.

LOSS OF BODY WATER AFTER LESIONS OF THE HYPOTHALAMUS. It has been clearly shown by Camus and Roussy (4) and by Bailey and Bremer (5) that a lesion of the hypothalamus, without damage to the hypophysis, is followed by diuresis. We herewith present data that the same is true in the bird as they have shown for the dog and cat.

TABLE 1

Solid excreta of normal and poikilothermous pigeons. Atmospheric temperature, 29 to 30°C. Body temperature, 39 to 42°C. Birds given water but no food

	STARVATION PERIOD	COLLECTION PERIOD	SOLIDS	AVERAGE WEIGHT SOLIDS PER 24 HOURS
	hours	*hours*	*grams*	*grams*
Normal	24	24	1.0	
	72	48	1.2	
	120	48	1.1	
	168	48	1.3	0.7
Normal	24	24	1.5	
	72	48	1.1	
	120	48	0.9	
	192	72	2.4	0.7
Normal	24	24	0.4	
	48	24	0.9	
	72	24	0.7	
	96	24	0.8	0.8
Poikilothermous	24	24	0.8	
	50	26	1.6	
	75	25	1.7	
	99	24	0.9	1.2
Poikilothermous	24	24	1.5	
	48	24	1.8	
	72	24	0.5	
	96	24	1.0	1.2
Poikilothermous	20	20	0.6	
	46	24	0.7	
	70	26	1.0	
	94	24	1.2	0.9

Methods. As previously pointed out (1), the physiological picture following decerebration in the pigeon varies according to whether the removal of the cerebral hemispheres is performed with or without damage to the thalamus. In this work the cerebral hemispheres were carefully removed with care to avoid deep traumatism of the thalamus; the hemor-

rhage controlled; and then with a clear view of the thalamus, the entire center was destroyed with an electro-cautery. Proper technique of decerebration is essential to procure the results described. Excessive bleeding, blood clots in the fourth ventricle or over the cerebellum may lead to death in a few hours without the appearance of these symptoms. The optic lobes and cerebellum need not be damaged. Forced movements, rigidity, muscle incoördination, etc., do not appear if the lesion does not extend posterior to the thalamus. Histological figures of such a decerebration have been published previously (1). It had been learned that a unilateral lesion of the dorsal parts of the thalamus, or a bilateral dorsal thalamic lesion, does not abolish the ability to regulate body temperature (6), but in order to destroy the temperature regulating centers the inferior parts of the thalamus must also be damaged. This is a confirmation in the bird of the original experiments of Isenschmid and Krehl (7) who, working with the rabbit, found the basal thalamic centers essential for temperature regulation. In the bird, destruction of the thalamus does away with temperature regulation and causes diuresis.

In certain birds the diuresis following the thalamic lesion is a very conspicuous thing. Normally under the standard conditions, the excreta of the pigeon contains very little water. That a diuresis rather than a diarrhea might be the cause of the rapid loss of body weight, was first apparent when, using fasting birds, it was observed that at times the paper covering the bottom of the cage looked as if it had been dipped in water. In certain birds (nos. 75 and 49, table 3) this gross diuresis was evident on simple observation.

To measure the diuresis and loss of body water, two methods have been employed. First, collection of the urine in a metabolism cage; and second, by determinations of the loss of body weight.

The first method is not a satisfactory one since the amount of water lost from the body in the urine is very small as compared with that of mammals. Birds seem to be very efficient in their conservation of water. However, the attempt to collect the urine in a metabolism cage was made in several instances and the results are given in table 2. These values are minimal ones since it is impossible to prevent some loss by evaporation as the fluids pass over the surfaces of the collecting vessels. But the relative values for the normal and operated birds are reliable since the evaporation factor is equally applicable to both.

Determination of the water loss through the intestinal and urinary tracts does not of course determine the total water loss from the body. Loss of body weight however will measure the total water loss. This method has been used uniformly in this work. The justification of using loss of body weight to measure water loss in this work, lies in the following considerations.

a. Loss of body weight must be due to the loss of tissue substance, excreted gases and water. Since the animals are fasting, changes of weight due to intake of food and water are eliminated.

b. In the adult bird in the fasting condition the respiratory quotient lies between 0.70 and 0.75. The oxygen intake and the carbon dioxide output were measured and under the standard conditions maintained, the excess loss of weight due to carbon dioxide excretion is almost balanced by the weight of the absorbed oxygen. This factor is less than 2 grams per 24 hours.

c. The excess weight of protein catabolised in the operated birds as compared with the normal, when determined by the weight of excreted urinary and intestinal solids does not exceed 3 grams per 24 hours. In order to allow for the loss of weight due to metabolic changes in which the end products are substances other than water the following corrections are applied in the tabulated measurements. In the normal birds, 1.5

TABLE 2

Volume of urinary and intestinal water excreted by normal and poikilothermous birds as collected in metabolism cage. Body temperature 39 to 42°C. Atmospheric temperature 30°C. Birds fasting, no food or water for preceding 18 hours.

NUMBER OF BIRD	COLLECTION PERIOD	WATER COLLECTED	CALCULATED RATE PER 24 HOURS	LOSS OF BODY WEIGHT	CONDITION OF BIRD
	hours	*cc.*	*cc.*	*grams*	
61	23	18	19	36	Poikilothermic
60	21	24	27	42	Poikilothermic
54	10	18	43	28	Poikilothermic
54	22	6	7	20	Normal
54	22	6	7	16	Normal

grams loss of weight is taken as the maximum excess of carbon dioxide elimination over the oxygen absorption through the twenty-four hour interval. Four grams loss of weight is taken as the maximum loss of protein. This value was obtained as follows: the total dry solids eliminated is not greater than 0.7 gram. Assuming this to be principally nitrogen, this value multiplied by 6.25 gives 4.6 grams as an average weight of dry protein catabolised. Six grams per 24 hours amply covers the loss of weight due to excretions of substances other than water. Similarly for the operated birds, a value of 6.5 grams is allowed for protein loss and 1.5 grams for carbon dioxide loss or a total of 8 grams per 24 hours. The tabulated determinations of water loss are thought to be minimal ones and may be slightly greater than those specified in the percentage columns. It is evident that such a determination of water loss from the body does not differentiate between water preformed in the body and water formed in the course of metabolic processes.

The excessive loss of water that follows decerebration and complete destruction of the thalamus of the pigeon is shown in table 3. As con-

TABLE 3

Loss of body weight and body water after destruction of the thalamus. No food or water for 24 hours preceding the decerebration. Atmospheric temperature, 30°C. Body temperature, 39 to 41°C.

NUMBER OF BIRD	BODY WEIGHT	TIME INTERVAL	LOSS OF BODY WEIGHT	CALCULATED WATER LOSS PER 24 HOURS	WATER LOSS PER 24 HOURS AS PER CENT OF BODY WEIGHT	LENGTH OF LIFE
		hours	*grams*	*grams*	*per cent*	
50	254	24	36	28	11	14 days
76	328	24	42	34	10	21 days
35	281	24	38	30	10	23 days
61	280	23	41	33	12	36 hours
53	278	18	31	34	12	24 hours
77a	297	24	46	38	13	24 hours
47	254	24	41	33	13	9 days
60	274	24	46	38	14	36 hours
75	334	11	34	64	19	24 hours
53	247	5	11	45	18	24 hours
49	286	16	48	64	22	16 hours
41	300	24	65	57	19	24 hours
77b	285	2	15	172	60	2 hours

TABLE 4

Water loss of normal pigeons and of normal pigeons anesthetized and cranium opened, but the cerebrum not removed, and the wound then closed. No food or water for 24 hours preceding the determinations

NUMBER OF BIRD	BODY WEIGHT	TIME	LOSS OF BODY WEIGHT	CALCULATED WATER LOSS PER 24 HOURS	WATER LOSS AS PER CENT BODY WEIGHT	CONDITION
	grams	*hours*	*grams*	*grams*	*per cent*	
26	322	24	15	9	3	Normal
27	260	24	15	9	4	Normal
27	267	24	17	11	4	Normal
54	309	24	17	11	4	Normal
48a	339	24	22	16	5	Normal
32	270	20	20	18	7	After anesthesia
48b	273	24	18	12	4	After anesthesia
52	278	21	17	13	5	After anesthesia
16	278	24	20	14	5	After anesthesia

trols on these findings, the loss of water in normal fasting pigeons is shown in table 4. As a further control, the water loss was determined in a series of normal fasting birds which were etherized, the cranium trephined,

the dura incised and the wound then closed without further damage to the brain. Such a proceeding eliminates the anesthetic as a possible cause of the diuresis.

It will be observed from these tables that the rate of loss of body water per 24 hours under standard conditions of normal birds and of normal birds subjected to etherization and trephining is from 4 to 7 per cent of the fasting body weight per 24 hours. The rate of loss of body water per 24 hours after decerebration and destruction of the thalamus is at the rate of 10 to 60 per cent of the body weight per 24 hours.

It will also be noted that the period of life after the operation is dependent on the extent of the resulting anhydremia. Such birds may be kept alive for several weeks if the initial loss of body water is at a rate not exceeding 14 per cent of total body weight per 24 hours. The longest period of life of such a bird in this series was twenty-three days. If the diuresis and resulting anhydremia reaches the value of about 18 per cent of the body weight death has always occurred in less than 36 hours. The significance of these dehydrations to disturbances of body temperature is considered in the subsequent report.

On the neurological side, the writer wishes to call attention to the fact that this diuresis does not follow simple decerebration if care is taken not to damage the thalamus. As is well-known the cerebral hemispheres can be removed without the diuresis here described and such birds may easily be kept for months without noticeable alterations of water elimination, metabolic disturbances or changes of the ability to regulate body temperature. In order to produce this diuresis the basal parts of the thalamus must be traumatised. The effect can be produced without traumatism of the hypophysis. It is not hypophyseal in origin unless it be conceived that the decerebration has interfered with the pathway of absorption of the hypophyseal hormones by way of the cerebro-spinal fluid, as suggested by Hering. However in the light of the results of Bailey and Bremer (5), this is an improbable assumption since they succeeded in causing a similar diuresis by a minute hypothalamic lesion without decerebration and with minimal disturbances to the third ventricle.

SUMMARY

Complete destruction of the thalamus in the bird is followed by excessive loss of body water and a marked diuresis. Under standard conditions of fasting and temperature, water is lost from the body after such a cerebral lesion at the rate of from 10 to 60 per cent of the fasting body weight per 24 hours as contrasted with the loss of water of normal birds of 4 to 7 per cent of the body weight, under similar standard conditions.

A water loss at a rate not exceeding 15 per cent of the body weight per 24 hours is compatible with life. If the rate of loss is 18 per cent or greater, the birds could not be kept alive for periods of more than thirty-six hours.

BIBLIOGRAPHY

(1) Rogers: Journ. Comp. Neurol., 1919, xxxi, 17.
(2) Rogers and Lackey: This Journal, 1923, lxvi, 453.
(3) Freund and Grafe: Pflüger's Arch., 1917, clxviii, 1.
(4) Camus and Roussy: Compt. Rendu. Soc. Biol., 1920, lxxxiii, 1587; 1922, lxxxvi, 1070.
(5) Bailey and Bremer: Arch. Int. Med., 1921, xviii, 773.
(6) Rogers: This Journal, 1919, xlix, 271.
(7) Isenschmid and Krehl: Arch. f. exper. Path., 1912, lxx, 109.

STUDIES OF THE BRAIN STEM

IX. On the Relation of Cerebral Puncture Hyperthermia to an Associated Anhydremia

FRED T. ROGERS

From the Physiology Laboratory, Baylor Medical College, Dallas

Received for publication February 18, 1924

In the preceding paper (1), experimental work is cited showing that after decerebration and destruction of the thalamus in the pigeon, but with minimal damage to the remainder of the brain, there constantly follows an excessive loss of body water. It is there shown that if the resulting dehydration does not occur at a rate exceeding 15 per cent of the body weight per twenty-four hours, the bird may be kept alive for several weeks, but an anhydremia occurring at the rate of 20 per cent of the body weight per twenty-four hours is incompatible with life.

Many workers have recently directed attention to the relations between the water content of the body and the regulation of body temperature. The writer's attention was directed to this relation by the finding that destruction of the thalamus in the pigeon leads constantly to the two effects of loss of ability to regulate body temperature and a diuresis. It was therefore of interest to determine if any related connection between the two conditions could be established.

After the destruction of the thalamus, the pigeon immediately becomes poikilothermic. After this operation it was found that many such birds could be kept alive for several weeks if they are kept in a warm incubator adjusted to a constant temperature of 29° to 31°C. Under such conditions the body temperature is artificially set at the normal level of about 40°C. This difference of 10°C. between atmospheric and body temperature is due to the protective insulation afforded by the feathers. In work earlier reported (2) on the temperature regulating centers it was stated that a true hyperthermia, or one occurring independently of the atmospheric temperature had not been observed following a brain lesion in the pigeon. From the point of view of cerebral physiology this was significant as hyperthermias following puncture of the corpus striatum have been frequently cited (3) as evidence that the corpus striatum is functionally related to the regulation of body temperature. Since the publication of this earlier report, a few instances have been found in which a hyperthermia of the bird may occur following a brain lesion.

Obviously a hyperthermia may result from either of two conditions. First, if the activity of the temperature regulating centers is impaired, so as to render the animal poikilothermic, a sufficient elevation of the atmospheric temperature will cause a rise of body temperature above the normal value. Such a hyperthermia will be dependent on the two factors of atmospheric conditions and the insulating efficiency of the hair or feathers. It does not seem to the author that these essential determining conditions have been fully appreciated by some of the workers on temperature centers, whereas on the other hand they have been carefully controlled in the recent work of Bazett and Penfield (4). A second type of hyperthermia is that in which the body temperature rises above the level determined by the combined influence of atmospheric temperature and the insulating action of the hair, fur or feathers. This latter type is here considered as a true hyperthermia and is the condition discussed in this paper.

Balcar, Sansum and Woodyatt (5) have stated that the injection of a sufficient quantity of hypertonic glucose solution intravenously, leads to dehydration and a rise in body temperature. Some question has been raised as to their interpretation of their results, that the resulting fever is a dehydration effect, by the work of Keith (6) who states that by the use of purified hypertonic saccharose solution a high degree of dehydration may be produced without a rise in body temperature. Keith states that a reduction of plasma volume of 24 to 44 per cent and a total water loss of 11 per cent of the body weight does not lead to fever. The results of the present work entirely agree with Keith's report, but they also indicate that if there occurs a still more severe loss of body water, about 19 per cent of the body weight, hyperthermia may follow.

Method of producing dehydration in the bird. The work was done with pigeons. Certain peculiarities of avian physiology render the bird a good test object for the study of this particular phase of temperature regulation.

In any studies of temperature regulation and brain lesions induced by surgical procedures in mammals, there is a constant uncertainty as to whether any resulting rise of body temperature may not be due to infection. As is commonly known, the pigeon is so resistant to infection by the ordinary organisms that this particular feature is eliminated. It is not necessary to even try to maintain aseptic conditions in the surgical work on the pigeon brain. The following crucial test was made on this question. Two pigeons were decerebrated without asepsis. In one case after the cerebral hemispheres had been removed, fresh saliva was dropped in the cranial cavity and the wound then closed. In the second case, fecal droppings were scraped from the floor of the cage, and put into the cranial cavity. Both birds were then kept for one week. There was

no subsequent rise of body temperature in either bird: at autopsy there were no signs of pus in the cranial cavity. In the bird a rise of body temperature can not then be attributed to infection. Furthermore, as will be seen from the tables, although no asepsis was maintained in any of the operative work, which has involved several hundred birds, hyperthermia has followed only in the specific cases here described.

Furthermore it should be clearly stated that since destruction of the thalamus renders the bird poikilothermic, it is easy to induce a rise of body temperature by simple exposure to an atmospheric temperature above 30°C. In this work, therefore, particular care has been taken in the regulation of atmospheric temperature conditions, and as will be observed in table 1 the hyperthermias have occurred with the atmospheric temperature constant at 30°C.

In the third place, it should be stated that the destruction of the thalamus does not lead to disturbances of muscular action, rigidity or incoördination, if the mid-brain is not traumatised. In the poikilothermic condition excessive muscular activity will lead to a rise in body temperature. Thus, in two instances in which this point was tested, the poikilothermic birds were forced to fly for fifteen to thirty minutes. Both birds promptly died with body temperatures of 44° to 46°C. Any bird therefore that exhibited excessive muscular activity or incoördination was rigidly excluded from consideration.

The three preceding items are emphasized, because it is obvious that under experimental conditions a hyperthermia can not be causally related to dehydration, unless it is shown that the rise of temperature is independent of infection, excessive muscular activity or atmospheric temperature variations.

If a pigeon is decerebrated and the thalamus and hypothalamus are destroyed with an electro-cautery, an excessive loss of body water always follows (1). The diuresis and total loss of body water after this operation may be from ten to twenty-five per cent of the body weight in twenty-four hours. If the total water loss from the body is at a rate of about 20 per cent of the fasting body weight, a hyperthermia frequently follows (table 1). By the decerebration method followed, hyperthermia following anhydremia was not always produced as in no. 49, table 1. There may therefore be certain conditions which will prevent the onset of fever after even severe dehydration. With the operative method employed it is not always possible to control hemorrhage from the basal cerebral and mesencephalic arteries. Excessive bleeding and blood clots over the cerebellum and in the fourth ventricle may lead to death without hyperthermia as in the case of this bird.

Making all due allowance for all the preceding conditions, it will be observed from table 1 that a hyperthermia *can* be induced in the bird by

a cerebral lesion, and is independent of excessive muscular activity, variations of atmospheric temperature or infection, but which is associated with a loss of water from the body at a rate of not less than 18 per cent of the fasting body weight per twenty-four hours.

The water loss has been routinely calculated in terms of the loss of body weight per twenty-four hours. Comparison of the curves of figure 1 suggests that the essential feature for the onset of fever is not the absolute weight of water lost, but the rate of water loss. Thus it will be seen that in pigeon 77a a severe water loss of about 50 grams over a period of thirty-four hours did not cause a rise in body temperature, but in pigeon

TABLE 1

Relations between hyperthermia and the degree of loss of body water after destruction of the thalamus. Birds kept at atmospheric temperature of 30°C. No food or water for 24 hours preceding the cerebral operation

NUMBER OF BIRD	BODY WEIGHT	WATER LOSS PER 24 HOURS	WATER LOSS CALCULATED AS PER CENT BODY WEIGHT	MAXIMUM BODY TEMPERATURE	REMARKS
	grams	*grams*	*per cent*	°C.	
50	254	28	11	41.5	Lived 14 days
76	328	34	10	39.0	Lived 21 days
47	254	33	13	41.0	Lived 9 days
60	274	38	14	40.5	Lived 36 hours
75	334	64	19	44.0	Lived 24 hours
53	247	45	18	44.2	Lived 24 hours
49	286	64	22	40.5	Lived 16 hours
41	300	57	19	44.6	Lived 24 hours
77b	285	172	60	46.1	Lived 2 hours

The method of measuring the water loss is described in the preceding paper. If necessary, records similar to those of the first four birds of this table could be given for a large number of decerebrate birds. Of a large number of decerebrations, hyperthermia has been observed only in those birds whose records are here given.

54 with a water loss of 38 grams in ten hours, and in pigeon 77b with a water loss of 16 grams in two hours, fatal hyperthermia ensued. It seems to the writer that insofar as the water exchange can be related to temperature regulation it will probably be found that under normal conditions, the *rate* of water loss or exchange is the crucial feature. This may account for the discrepancy in the reported results of Woodyatt and Keith. Thus Woodyatt states that in his work the rate of injection of hypertonic glucose solution was 15 grams per kilogram body weight, and Keith states that in his work the rate of injection of saccharose was 8 grams per kilo per hour. Furthermore, Woodyatt states that the injection of glucose at the rate of 10 grams per hour did not cause fever.

The writer also wishes to call attention to another feature in which the present results harmonize with those of Balcar and Woodyatt. They state that it was possible to produce fever in the poikilothermic dog. The birds here described are poikilothermic and hyperthermia follows a sufficient degree of dehydration.

After it had been observed that hyperthermia sometimes followed the cerebral lesion if the loss of body water was sufficiently rapid and extensive,

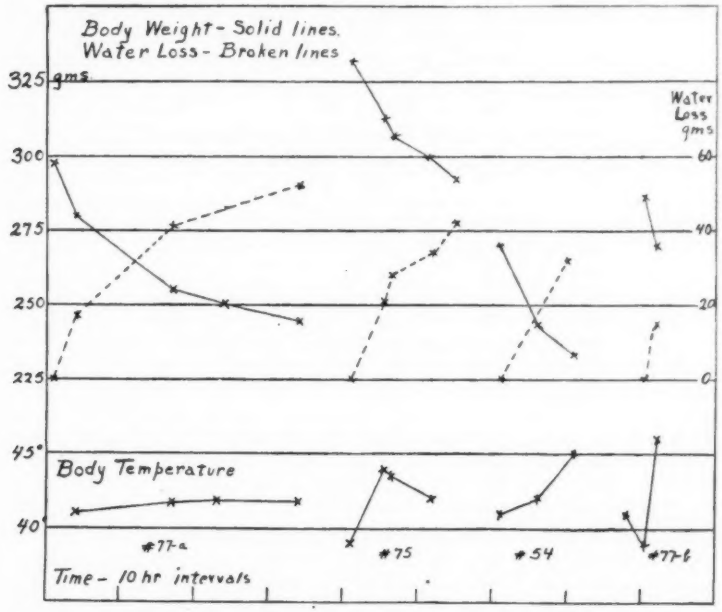


Fig. 1. The relations of quantity and rate of loss of body water to the body temperature of pigeons immediately following destruction of the cerebral hemispheres and thalamus. The loss of body weight is shown by solid lines and the weight of the water lost by diuresis and the respiratory tract, is shown by the broken lines. It will be observed that hyperthermia frequently occurs if the water loss is sufficiently rapid.

it then became apparent that if the rise in temperature was dependent on the water balance, and not an unrelated accident, it should be possible to more certainly produce it by water deprivation preceding the cerebral operation. The preceding considerations however indicate that there are certain precautions to be taken. First, simple prolonged starvation of the bird, as previously shown (2), leads to the gradual onset of a general depression of the bird with the temperature becoming subnormal. If such a condition of depression ensues before the cerebral lesion is made,

negative results might be anticipated. If on the other hand the period of starvation is too brief, and the cerebral lesion causes only a mild diuresis, negative results might follow. The desirable condition would be

TABLE 2

Dehydration and hyperthermia after water starvation and destruction of the thalamus. No food or water for 24 hours preceding the initial measurements.

NUMBER OF BIRD	STARVATION PERIOD	BODY WEIGHT	BODY TEMPERATURE	ATMOSPHERIC TEMPERATURE	WATER LOSS	WATER LOSS AS PER CENT BODY WEIGHT
	hours	*grams*		*°C.*	*grams*	
54		309	42.0	29–31		
	70	265	41.0		43	8.1
	Destruction of thalamus followed by diuresis					
	73	243	42.0	30	47	15.2
	78	233	45.0	30	56	18.1
99		306	42.0	20–25		
	48	275			19	7.0
	99	254	42.5		27	8.8
	126	244	41.0		30	9.8
	144	238	39.5		32	10.4
	Destruction of thalamus followed by diuresis					
	150	224	40.0	30	44	14.3
	156	217	43.5	30	49	16.0
98		306	42.5	20–25		
	48	273	41.8		21	6.9
	126	237	39.3		38	12.4
	142	229	39.0		41	13.4
	Destruction of thalamus followed by diuresis					
	150	213	40.3	30	45	14.7
	154	208	42.0	30	57	18.6
	157	206	41.0	30	60	20.0
	165	195	38.5	30	68	22.2
97		310	41.8	20–28		
	96	264	39.3		40	13.0
	144	242	39.5		62	20.0
	Destruction of thalamus not followed by severe diuresis					
	150	235	39.3	30	67	21.6
	166	222	39.3	30	80	25.8
100	Died 2 hours after operation. Figures therefore not given					

to expose the fasting bird to a warm, dry atmosphere so as to produce a rapid loss of water within a few days, and then the cerebral lesion with its resulting diurésis would be expected to lead to hyperthermia, *if* indeed the rise of temperature were causally related to the resulting anhydremia.

The results of such a test are given in table 2 and figure 2. Five adult pigeons were deprived of food and water for four to six days and then the thalamus and cerebral hemispheres destroyed. Pigeon 100 died two hours after the operation from intracranial hemorrhage. Pigeons 54 and 99 exhibited severe hyperthermia followed by death. In the case of no. 98 the body temperature rose 3°C. above the fasting level, and in one case neither severe diuresis nor fever occurred.

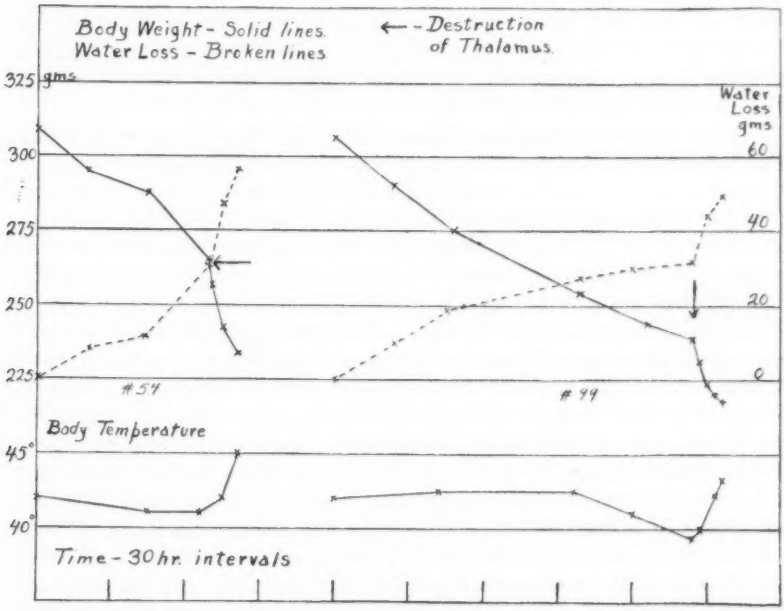


Fig. 2. Hyperthermia induced by water starvation for 3 to 5 days followed by decerebration and destruction of the thalamus. The body weight is indicated by solid lines, the total loss of body water by broken lines. It will be observed that destruction of the thalamus in these cases was followed by the two concomitant effects of a rise of body temperature and severe dehydration.

In this crucial test it is shown that in three out of five attempts, hyperthermia could be induced by sufficient water starvation followed by destruction of the thalamus and resulting diuresis.

Hyperthermia and anhydremia without cerebral lesion. It is well known that water deprivation, when exposed to desert conditions of a hot dry atmosphere, may lead to anhydremia and fever (7). It would be expected that exposure of any homothermous animal deprived of water to a hot, dry atmosphere will lead to similar conditions.

If the preceding conclusion is correct, that the hyperthermia following the cerebral lesion is due to the exhaustion of the water reserves of the body, and that this reserve is about 18 per cent of the fasting body weight, it is then of interest to compare these findings with those resulting from simple exposure of normal fasting pigeons, deprived of water, to a warm dry atmosphere. This was done on three pigeons. After a preliminary fasting period of twenty-four hours, they were exposed to a dry atmosphere maintained at the body temperature of the bird, 40° to 42°C. After two to three days the body temperature reached 44° to 45°C. and the birds died. Calculation of the amount of water lost at the time of death for the three birds gave values of 16 per cent, 18 per cent and 19 per cent of the initial fasting body weight.

It will be noted then that the rapid loss of 16 to 20 per cent of the body weight as water, by exposure to a warm, dry atmosphere, leads to hyperthermia in normal pigeons, just as happens to decerebrate birds which exhibit an acute, severe diuresis.

Discussion. The preceding results should now be considered with respect to their application to the problems of the mechanism of body temperature regulation.

In the first place, from the viewpoint of cerebral physiology, it is to be noted that hyperthermia following a brain lesion may be induced independently of supposed centers in the corpus striatum. Complete removal of both corpora striata, as previously pointed out by Moore (8), and by the present writer (2), does not necessarily lead to disturbances of temperature regulation. On the other hand the complete destruction of the thalamus abolishes temperature regulation in all homothermous animals which have been studied. Thus Isenschmid and Krehl (9) first definitely proved this for the rabbit. Bazett and Penfield (4) and Dusser de Barenne (10) have shown this is true of the cat; the present writer (2) has shown the same for the pigeon; and in decerebrate dogs which have been kept alive for long periods of time (Goltz) the ability to maintain temperature regulation was preserved and autopsy showed the medial and basal thalamic nuclei intact (11).

In the second place, it is here shown that in the bird under certain conditions of water deprivation and anhydremia, hyperthermia can be induced irrespective of the possibility of the fever being a result of bacterial contamination.

Such a hyperthermia is not easily induced. Consideration of the tabulated findings will show that in order to cause the dehydration fever in the bird there is a factor of safety in the water supply of the body of about 18 per cent of the total body weight, which must first be exhausted. To the writer it seemed very significant that the minimum level of water loss whereby anhydremic fever may be induced in the bird is about 20

per cent of the fasting body weight, for similar degrees of dehydration associated with fever may occur clinically in severe diarrheas (7) although in these cases the factor of bacterial infection can not be excluded as a factor in causing the accompanying fever.

In the third place, although it is shown that an anhydremic fever can be produced, it is obvious that a relation of water loss to fever in its common clinical manifestations is not thereby established. However, evidence is cited indicating that the essential causative feature in producing this anhydremic fever is not the absolute quantity of water lost, but rather the *rate* at which it is lost. A relatively small quantity of water lost quickly may cause a rise in temperature in the experimental work here described, while a great water loss at a slow rate, as in starvation, does not lead to fever. This factor of *rate* of water loss as well as the absolute quantity of water loss or exchange, *may* be significant in studies of temperature regulation and the causation of fever.

Again, it is to be noted that even severe diuresis, alone, does not necessitate a subsequent rise of body temperature. Attention is directed to the experimental fact that in the hyperthermic birds not only was there a great loss of body water preceding the onset of fever, but that no water was given after decerebration. It is commonly known that diabetes insipidus is not of necessity associated with fever; and in the experimental polyurias described by Bailey and Bremer (12), hyperthermia did not uniformly occur. It is evident that the loss of water may be entirely or to a large extent counteracted by permitting an ample intake.

Furthermore, although facts are presented which indicate a parallelism between certain hyperthermias and severe dehydration, the writer is unable to see, as has been suggested by Woodyatt, that the theory of the nervous regulation of body temperature should be abandoned. The water balance is only one of the factors in temperature regulation. Certainly all the experimental work on the homothermous animals indicates the thermogenic factor of increased muscular activity in response to atmospheric cold, and the thermolytic respiratory mechanism demand that the basal parts of the brain below the corpus striatum be functionally intact. And as is suggested by Barbour (13) even the water shifting processes are probably related to some nervous control, the details of which are at present unknown.

SUMMARY

Hyperthermia may be induced in the pigeon by decerebration and destruction of the thalamus, if the hypothalamic lesion leads to a sufficiently severe diuresis and a resulting anhydremia.

If the pigeon has been previously deprived of water, this result is more easily obtained.

Evidence is cited that the hyperthermia, if not causally related to, is at least paralleled by a dehydration of not less than 16 to 20 per cent of the fasting body weight. Dehydrations of less than 15 per cent per twenty four hours did not lead to hyperthermia.

The water reserve of the pigeon is about 18 per cent of the fasting body weight. The rapid loss of this quantity of water from the body will lead to fever and death of both normal homothermous and decerebrate poikilothermous birds.

Attention is directed to the fact that this is a type of hyperthermia that may be induced by a cerebral lesion without involvement of presumed temperature regulating centers in the corpus striatum.

Evidence is offered, suggesting that so far as the water balance of the body and temperature regulation are related, the essential feature is the rate of water loss or exchange, rather than the absolute quantity of water loss.

BIBLIOGRAPHY

(1) Rogers: This Journal, 1924, lxviii, 499.
(2) Rogers: This Journal, 1919, xlix, 271.
(3) Howell: Textbook of physiology, 8th ed., Philadelphia, 1921.
(4) Bazett and Penfield: Brain, 1922, xlv, 185.
(5) Balcar, Sansum and Woodyatt: Arch. Int. Med., 1919, xxiv, 116.
(6) Keith: This Journal, 1923, lx, 394.
(7) Marriott: Physiol. Reviews, 1923, iii, 275.
(8) Moore: This Journal, 1918, xlvi, 253.
(9) Isenschmid and Krehl: Arch. f. Exper. Path., 1912, lxx, 109.
(10) Barenne: Arch. Neerland. de Physiol., 1919, iv, 31, 124.
(11) Holmes: Journ. Physiol., 1901, xxvii, 1.
(12) Bailey and Bremer: Arch. Int. Med., 1921, xviii, 773.
(13) Barbour: Physiol. Reviews, 1921, i, 295.

THE INFLUENCE OF PREGNANCY AND LACTATION ON THE WEIGHT OF ADRENAL GLANDS IN THE ALBINO RAT

JOHN C. DONALDSON

From the Department of Anatomy, School of Medicine, University of Pittsburgh

Received for publication February 18, 1924

The association of the activities of the adrenal glands with those of the reproductive system has been recognized for a number of years. The most recent work on the subject is a study by Riddle (1) on pigeons. He shows convincingly that ovulation in this form is accompanied by a marked increase in the weight of the adrenal glands. In this paper he also reviews briefly the literature on the subject. In the case of mammals it appears that aside from scattered and conflicting notes on man, the dog and the rabbit, accurate observations have been recorded on the size or weight of the adrenals during pregnancy and lactation in the guinea pig. Guieysse (2), Verdozzi (3) and Castaldi (4) all agree that a marked increase takes place during this period. Observations on a short series of rats by Herring (5) showed a similar but much less marked increase in adrenal weight. The above work indicated the desirability of a further examination of the weight of the adrenal glands in rats during pregnancy and lactation.

The animals used were from the colony of albino rats (Mus norvegicus albinus) started in 1919 in the Department of Anatomy from animals obtained locally. No new strains have been introduced since. The colony is fed a mixed diet, consisting of fresh table scraps and corn. These rations are not weighed. The rats grow and breed well under these conditions of food supply. Wooden cages patterned after the Wistar type are used. During the experiments the pregnant-lactating and the control animals are placed in the same or adjacent cages, so that food, light and temperature conditions are similar.

As will be seen from table 1, the morbidity rate is high. This may be associated with the fact that it is impossible to keep an even temperature in the rat-room particularly during the winter. The two types of pathological conditions most frequently seen are lung disease and infection of the middle ear and adjacent mastoid cavity.

The observations covered the period from early pregnancy (about the 6th day) to the close of lactation. All test animals were carrying or

suckling their first litter and the controls were nullipara. The body weights ranged from 110 to 241 grams, and the body lengths from 161 to 213 mm.

Each animal was killed with ether, weighed, measured for total and body length, and opened. The adrenal glands were at once removed, freed from surrounding fat and placed in a closed weighing bottle, to be weighed to 0.2 mgm. after the examination of the animal was completed. The abdominal viscera were then examined. If the animal was pregnant, both horns of the uterus were removed and weighed with their contents. This weight was subtracted from the total body weight of the animal, no correction being made for the weight of the uterus since this is negligible. This corrected body weight was used as the "body weight" in making all calculations. The embryos were removed from the uterus, freed from the membranes and their crown rump length measured. Their ages were estimated from their average length and average weight (6). The thorax of the rat was then opened and the heart and lungs examined for pathological conditions. The middle ear and mastoid bulla on each side were next opened and a record made of the presence or absence of pus. All these records were entered on a separate card for each rat.

Since the rats used were neither of the same body weight nor length, the weights of their adrenals could not be directly compared with one another. It was necessary therefore to use some standard to which the gland weights could be referred and by which their deviation could be measured. Such a standard is furnished by table 71, *The Rat*, (7), and the method for using it is described on page 4 of that book.

The steps in using this method are outlined as follows. An experimental animal is killed and weighed. The adrenals are removed and weighed. Table 71 is next consulted. This table gives the weights of the adrenal glands in rats for different body weights and different body lengths, from birth to middle age. The value for the adrenals in an animal having the same body weight as the test animal is obtained from this table. This value we call the "expected weight" of the adrenals for a given body weight. The difference between the weight of the adrenals observed in the test animal and the expected weight as given in table 71 is then expressed as a percentage of the expected weight. The final observation would read, for example, "rat, no. 10, adrenal weight, + 5 per cent," meaning that in rat 10 the adrenals are 5 per cent in excess of the "expected weight" for rats of the same body weight as given in reference table 71. Since the Wistar tables give us a set of normal values for the adrenal weights throughout life, variations from these values, when expressed as a percentage, represent a given deviation from this norm regardless of the size of the animal. These percentage figures are then directly comparable and may be termed the percentage deviations for the adrenals.

The use of the Wistar tables does not involve accepting them as the only normal values possible. It does recognize them, however, as establishing a growth curve for the gland that can serve as a basis for comparison.

The percentage deviations for 80 pregnant or suckling females and 76 controls were thus obtained. The test and control animals fall at once into two main groups, those free from visible signs of disease and those showing evidence of injury or infection. The former are designated in the tables as "healthy" and the latter "pathological." The grouping of the rats under these heads resulted in scattering the observations for the different litters, so that comparisons could not be made strictly between members of the same litter, as was originally intended. In each group the percentage deviations of the adrenals were averaged and entered in column 3 of table 1. The probable error of the mean was also calculated.

Healthy rats. Table 1 shows that the average of the adrenal weights in the 29 healthy controls deviated less than 3.0 per cent from the Wistar values. The adrenal weight average in the 29 healthy pregnant-suckling animals is 0.8 per cent below the weight of the controls. The probable error of the mean indicates that this difference between the groups has statistically no significance. In fact under conditions in our colony, we would be well pleased if two groups of normal animals showed average adrenal weights which agreed as closely. The evidence, therefore, leads to the conclusion that there is no change in the adrenal weight of the healthy albino rat in the pregnancy-lactation period.

One possible explanation of this might be that large increases in the weight of the glands during one part of this period would be balanced by a decrease at another time. Table 2 indicates that this is not the case. In making this table, the 44 days from conception to the close of lactation were divided into quarters and the average adrenal weights for each quarter calculated, using the data for the healthy pregnant and lactating animals. Variations are to be expected but it is clear there is little fluctuation, the maximum difference being 5.9 per cent, a figure of little significance with so few observations in each group.

The last column in table 2 represents Verdozzi's (3) figures based on 35 cases. The pregnancy-suckling period of the guinea pig has likewise been divided into quarters. Castaldi (4) examined eleven pregnant guinea pigs and his figures agree with Verdozzi in showing a marked increase in adrenal weight in the first quarter followed by a loss of part of this increase in the second quarter. It is to be noted that in the rat also the second quarter shows the lowest value for the adrenal. The striking point, however, is the small fluctuation in the adrenal of the rat as compared with the marked fluctuations occurring in those of the guinea pig.

TABLE 1

Percentage deviations in the weight of the adrenals—albino rats. Based on body weight. Values in table 71 (loc. cit.) used as a standard

GROUPS	NUMBER OF CASES	MEAN BODY WEIGHT	MEAN WEIGHT OF BOTH ADRENALS	MEAN OF PERCENTAGE DEVIATIONS	PROBABLE ERROR OF THE MEAN OF THE PERCENTAGE DEVIATIONS
		grams	*mgm.*		
Healthy controls	29	163	44.7	2.67	±1.87
Healthy pregnant and lactating	29	167	45.3	1.86	±2.34
Pathological controls	47	174	47.9	6.4	±1.89
Pathological pregnant and lactating	51	163	57.8	11.8	±1.98

TABLE 2

Percentage deviations in the weight of the adrenals, in the four quarters of the gestation-lactation period. Determined as in table 1

	NO. OF CASES	(A) MEAN PERCENTAGE DEVIATIONS FOR ALBINO RAT	(B) CORRESPONDING DEVIATIONS FOR THE GUINEA PIG, VERDOZZI (3)
1st quarter (1–11 days)	9	4.9	20
2nd quarter (12–21 days)	12	−1.0	7
3rd quarter (22–32 days)	4	2.9	40
4th quarter (33–44 days)	4	4.1	70

TABLE 3

Percentage deviations in the weights of the adrenals—albino rats. Based on body length. Determined as in table 1

	NO. OF CASES	MEAN BODY LENGTH	MEAN WEIGHT OF BOTH ADRENALS	MEAN OF PERCENTAGE DEVIATIONS	PROBABLE ERROR OF THE MEAN OF THE PERCENTAGE DEVIATIONS
		mm.	*mgm.*		
Healthy controls	29	181	44.7	6.5	±1.79
Healthy pregnant and lactating	29	183	45.3	7.8	±2.37
Pathological controls	47	187	47.9	8.1	±1.84
Pathological pregnant and lactating	51	183	57.8	15.8	±2.45

In the mole there is a marked increase in the size of the adrenal during the active stage of the oestrous cycle (8) (9). Riddle (1) points out that any comparison in mammals between the adrenal weights in pregnant and non-pregnant animals must take into consideration the possibility of such weight changes associated with the oestrous cycle. A group of rats was studied with this in mind. There were 17 rats in the active phases of the oestrous cycle and 12 in the resting stage (10). The difference in deviation of the average adrenal weights between the two groups was less than 0.2 per cent. The series is short and more work is being done on this phase of the matter but the results so far obtained indicate that the oestrus has strikingly little influence on the weight of the adrenal glands in the rat.

Another possible source of variation is that in pregnancy and lactation we may be dealing with animals that are either markedly under or over weight as compared to their length, due to their physiological condition. By using body length instead of body weight as our standard of comparison, variations due to temporary changes in weight would be eliminated. This was done for the entire series and the results are shown in table 3. The adrenal weight in the healthy pregnant-lactating animals is here 1.3 per cent above that for the controls. This again is a very minor variation. These figures agree with those of table 1 in showing no significant increase in the weight of the adrenal glands in healthy rats during pregnancy and lactation.

These results are in contrast with those of Herring (5). In his series of nine rats examined at parturition, there was a 12 per cent increase in adrenal weight over litter controls. Two of the pregnant animals had very large glands, which served to raise the average, but after allowing for these there was still a distinct increase. Herring conducted careful autopsies and states that these rats were healthy so that infections of the lungs would have been observed. Infections of the middle ear, however, might have been overlooked unless especially searched for. On this point there is no statement in his paper. This condition influences the weight of the adrenal glands as is shown in the following section, and may explain the difference in results in the two series.

Pathological rats. The values for the pathological groups are interesting. Taken by themselves the figures in tables 1 and 3 showing enlargement in the diseased group would not be conclusive. However, a similar increase in adrenal weight in rats suffering from disease was noted in this colony in two other groups of albino females. This is in agreement with similar observations made by workers at the Wistar Institute (see also Riddle (1)).

The difference between the pathological controls and pathological pregnant-suckling animals is suggestive. The difference in deviation is close to three times the probable error of the mean, which makes it highly

unlikely that this difference is accidental. It occurs, whether body weight or body length is used as a standard of comparison (tables 1, 3). The evidence indicates that when the strain of pregnancy is added to that of disease an increase in adrenal weight does occur. It suggests also a possible source of error in observations of this kind, on the rat at least, since if we take the healthy and pathological rats together and include all the figures for the controls in one group and all those for the pregnant-suckling animals in another, the results would show a distinct adrenal enlargement in the latter. The percentage values would be 4.8 per cent for the controls and 8.6 per cent for the pregnancy-lactation group. This would not be a correct measure of the difference in deviation occurring in pregnancy and lactation because of the error introduced by the reaction of the adrenals to disease.

The observations show that the adrenals in healthy rats during pregnancy react differently from those of the guinea pig. This seems to fit in with the well-known fact that the rat can survive the removal of both adrenals and with the more recent observation by Lewis (11) that complete adrenalectomy in rats does not interfere with reproduction. He obtained litters from pairs of completely adrenalectomized animals.

This difference in response to similar physiological conditions in such closely allied species as the rat and the guinea pig emphasizes the danger of attempting to generalize at present in the field of endocrinology.

CONCLUSIONS

1. No increase in weight of the adrenal glands during pregnancy and lactation occurs in normal albino rats free from visible signs of disease.

2. An increase in weight of the adrenal glands occurs in unmated rats suffering from gross pathological lesions represented by lung infections and infections of the middle ear.

3. A further increase in weight of the adrenals occurs during pregnancy in rats suffering from pathological conditions.

BIBLIOGRAPHY

(1) Riddle: This Journal, 1923, lxvi, 322.
(2) Guieysse: Journ. d. L'anat. et de la Physiol., 1901, xxxvii, 312, 435.
(3) Verdozzi: Arch. Ital. de Biol. (Pisa), 1917, lxvi, 121.
(4) Castaldi: Arch. di Fisiol. (Florence), 1922, xx, 33.
(5) Herring: Brit. Med. Journ., 1920, ii, 886.
(6) Stotsenberg: Anat. Rec., 1915, ix, 667.
(7) Donaldson: The rat. Memoirs, Wistar Inst. of Anatomy, Philadelphia, vi, 1915.
(8) Kolmer: Arch. f. Mikro. Anat., 1918, xci, 1.
(9) Watson: Abstract of communication to the XI Internat. Physiol. Congress, Edinburgh, 1923.
(10) Long and Evans: The oestrous cycle in the rat and its associated phenomena. Memoirs, Univ. California, 1923, vi.
(11) Lewis: This Journal, 1923, lxvi, 503.

ON GLOMERULAR FILTRATION

H. L. WHITE

From the Physiological Department of Washington University, St. Louis

Received for publication February 21, 1924

The question as to whether the passage of fluid across the glomerular membrane is a process of filtration comparable to the passage of fluid across an inanimate membrane or is effected by a true secretory activity on the part of the membrane is an old one. In discussing this question one must be careful to distinguish between proof and presumptive evidence.

Unqualified acceptance of the filtration theory must be reserved until positive proof is presented that the excess of pressure in the glomerular capillaries over that in the glomerular capsule exceeds the osmotic pressure of the plasma colloids at a time when urine is being secreted. Up to the present time such proof has not been presented although its establishment seems to be tacitly assumed by most physiologists. It may be said that the attitude taken by the majority of workers is that the burden of proof rests on those who deny glomerular filtration.

Starling (1) showed that the osmotic pressure of the serum colloids was about 30 mm. Hg. Subsequent determinations by various workers of colloidal osmotic pressure of mammalian plasma have given figures averaging about 35 mm. Hg. Advocates of the filtration theory are therefore required to present evidence that the pressure in the glomerular capillaries is somewhat more than 30 to 35 mm. Hg. The presumptive evidence which has been advanced, i.e., the minimum arterial pressure compatible with urine secretion, the relation of the pressure in the obstructed ureter to arterial pressure during urine secretion, the relation of the extent of dilution of plasma colloids to the minimum arterial pressure compatible with urine secretion, etc., is familar to all physiologists. The fact that all this is merely presumptive evidence and not proof, however, has frequently been overlooked. An argument of Starling's (2) proposed to show that the pressure in the glomerular capillaries must be considerably greater than that in capillaries in general may be considered here. It is based on the pressure relations obtaining in a fluid passing through a tube from a segment of small bore to one of larger bore. The total energy of a mass m is represented by the equation $E = \frac{1}{2} m v^2 + \frac{m p}{d} + mgh$, i.e., the sum of the kinetic and potential energy. As the fluid passes from the segment of

small bore into that of larger bore the velocity and therefore the kinetic energy falls. Since the fall in total energy is a continuous one the potential energy or energy of pressure will gain at the expense of the kinetic energy or energy of velocity. As Starling points out, if one assumes that the diameter of the capillary bed in the glomerulus is 20 times that of the afferent artery the kinetic energy of the blood in the glomerular capillaries is only $\frac{1}{400}$ that in the afferent artery. Whatever fall in kinetic energy occurs, neglecting the gradual and continuous loss by friction of total energy, will be exhibited as an increase in potential energy or energy of pressure. The statement is therefore made that "Although therefore the pressure in the ordinary capillaries of the body is probably not greater than 20 to 30 mm. Hg, the glomerular capillaries might present a pressure little inferior to that in the main arteries of the body."

Before accepting this statement, however, let us inquire more closely into the relative magnitudes of the kinetic and potential energies possessed by the blood in the afferent artery. It is obvious that the blood in the glomerular capillaries cannot gain any more potential energy than the amount of kinetic energy lost in passing from the afferent artery to the glomerular capillaries. In order to be perfectly fair we shall assume a maximum figure for the velocity of the blood flow in the afferent artery. Volkmann (3) gives 56 mm. per second as the average velocity of blood flow in the metatarsal artery of the horse. The afferent artery to the glomerulus in the dog or man is certainly much smaller than the metatarsal in the horse and consequently the blood passing through it must have a much slower velocity. However, we shall assume an average velocity of 50 mm. per second which is certainly a maximum figure. Let us now calculate the relative magnitudes of the kinetic and potential energies of the blood stream at this point.

In the total energy equation we may neglect the factor mgh due to energy of position, which is the same in the afferent artery and glomerular capillaries. In order to give every possible chance for the kinetic energy factor to appear as large as possible we shall assume that all the kinetic energy is transformed into pressure energy when the blood enters the glomerular capillaries. Then, neglecting the loss by friction of total energy we have $\frac{1}{2} m v^2 + \frac{m p_1}{d} = \frac{m p_2}{d}$ where p_1 and p_2 are the pressures in the afferent artery and glomerular capillaries. Then $\frac{1}{2} m v^2 = \frac{m (p_2 - p_1)}{d} = \frac{m P}{d}$ where P is the difference between p_2 and p_1, i.e., is the increase in pressure due to the transformation of kinetic energy into pressure energy. Cancelling m and taking d, the specific gravity of blood, as unity, gives $\frac{1}{2} v^2 = P$. In other words we have converted the energy factor, $\frac{1}{2} m v^2$, into a force factor and the increase in pressure in the glomerular capillaries is determined by the kinetic energy lost. If we take v as 5 cm. per

second, $P = 12.5$ dynes / sq. cm. By the familar equation $p = hdg$ we find that 1 mm. Hg head of pressure corresponds to $\frac{1 \times 13.6 \times 980}{10} = 1332$ dynes / sq. cm. Therefore 12.5 dynes / sq. cm. $\equiv$ 0.009 mm. Hg. It is thus seen that the increase in pressure in the glomerular capillaries due to the transformation of kinetic into potential energy cannot be more than 0.009 mm. Hg which is of course negligible. This argument in favor of a high pressure in the glomerular capillaries is therefore not valid.

The work of Erlanger and Hooker (4), of Hooker (5) and of Gesell (6), (7), in which they demonstrate the relation of pulse pressure to urine formation is strong evidence in favor of the filtration theory. It is, however, as these workers of course recognized, only presumptive evidence and not proof. The work of Richards and Plant (8) is the nearest to a direct proof of glomerular filtration which has yet been advanced. The work is not, however, incapable of other interpretation. Their statement, "The results are believed to constitute direct evidence in support of the filtration hypothesis of glomerular function," is a refreshing departure from the unwarrantedly dogmatic assertions sometimes found in authors' conclusions.

Woodland (9) in a very long and involved paper which does not seem to have received the attention which it merits, concludes that in the frog no fluid passes the glomerular membrane either by filtration or secretion. He believes that the glomerular capillaries are merely a *rete mirabile*, acting to lower the blood pressure before the blood enters the tubular capillary system. The urine, both water and solids, is secreted entirely by the tubules. A critical discussion of his paper would lead us too far afield but his work should not be overlooked when the question of glomerular function is being considered.

Leonard Hill and McQueen (10) have denied the possibility of glomerular filtration on the grounds that the pressure in the glomerular capillaries is less than the osmotic pressure of the plasma colloids. They present a theoretical discussion of the pressure relations obtaining in the renal circulation of the mammal and also offer experimental results obtained with the frog's kidney. It is with the latter phase of their paper that we are concerned here.

Hill observed directly the glomerular capillaries while they were being subjected to an air pressure. He found that "a pressure of 5 to 10 mm. Hg suffices to slow distinctly the flow in the glomeruli." His conclusion is "that pressure in the glomerular capillaries is a low one, and that physiological permeation—not filtration—controls the passage of fluid through the glomeruli." Apparently this conclusion is reached because the pressure in the glomerular capillaries is presumably lower than the osmotic pressure exerted by the plasma colloids. Hill fails, however, to consider two very

important points. In the first place the glomerular capillary pressure of 5 to 10 mm. Hg was that observed under the conditions of his experiments. Since the operative procedure was rather severe it is quite probable that the glomerular capillary pressure in the normal frog is somewhat higher than the pressure as he observed it. In order for his objection to the filtration theory to be valid he should have shown that the glomerular capillary pressure *at a time when urine was being secreted* was less than the osmotic pressure of plasma colloids.

This leads us to the second point, the consideration of which forms the basis of the experimental work reported in this paper, namely, the osmotic pressure of the plasma colloids of the frog. Advocates of the filtration theory need only show that the glomerular capillary pressure is greater than the osmotic pressure of plasma colloids, if the assumption is made that the pressure in the glomerular capsule is atmospheric. Hill showed that the glomerular capillary pressure of the frog was quite low, say 10 mm. Hg on an average. It is quite true that if this is the normal glomerular capillary pressure in the frog and if the osmotic pressure of plasma colloid in the frog is about 30 mm. Hg as in the mammal, this combination of facts would deal a death blow to the filtration theory, at least in the case of the frog. But Hill did not determine the osmotic pressure of the plasma colloids in frog. The tacit assumption was made that it stands somewhere higher than 10 mm. Hg and therefore cannot be overcome by the filtration head of pressure existing in the frog's glomerular capillaries. That this assumption is not warranted will be shown by the results of determinations we have made of the osmotic pressure of frogs' plasma colloid. It is found that the osmotic pressure of frogs' plasma colloids is much lower than is the case with mammals (about 10 mm. H_2O) so that even if we accept Hill's figure of 10 mm. Hg as the glomerular capillary pressure in the frog there is still an excess of intracapillary pressure over plasma colloid osmotic pressure adequate to account for a process of filtration, provided always the assumption is made that the pressure in the glomerular capsule is atmospheric. The question as to whether or not this assumption, which is practically universally accepted by physiologists today, is justifiable will not be discussed in this paper.

METHODS. Large bull frogs of the species Rana catesbiana were used. These animals were in an excellent state of nutrition and there is no reason to suppose that their plasma protein content was below normal. They came from Louisiana and were caught in January. In the first experiment the approximate isoelectric point of the plasma proteins, the pH, osmotic pressure and protein content were determined. In subsequent experiments only the osmotic pressure and protein content were determined. It was thought advisable to determine the pH of the plasma and approximate isoelectric point of the plasma proteins on the basis of the following con-

siderations. Loeb (11) has shown that protein in an electrolyte solution exists as a metal proteinate on the alkaline side of the isoelectric point. The osmotic pressure is at a minimum at the isoelectric point and increases as the pH rises or falls from this point. Within a pH range close to the isoelectric point small changes in the pH cause considerable changes in the osmotic pressure, while within a pH range between 7 and 8 the slope of the curve obtained by plotting the osmotic pressure of albuminate solutions as ordinates against pH as abscissae is much less steep. It seemed advisable, then, to determine the pH of the plasma and the approximate isoelectric point of the plasma proteins. Because if it is found that the pH of the plasma lies at such a distance from the isoelectric point that the curve in this region is fairly flat, then we may be sure that small variations in pH, due to escape of CO_2 or to Donnan equilibrium effects, will not materially affect our osmotic pressure values. We may say here that such was found to be the case and it was therefore unnecessary to take precautions against loss of CO_2 in making osmotic pressure determinations. In fact it may even be said that whatever change in pH may occur as the resultant of the effect of the escape of CO_2 and Donnan effect is so slight that even if the curve of osmotic pressure plotted against pH were fairly steep in this region no material error would be introduced. This is because the change in pH inside the sac brought about by the Donnan effect opposes the change due to the escape of CO_2. The escape of CO_2 raises the pH while the membrane equilibrium effect lowers the pH on the protein solution side of a membrane when the protein exists as a proteinate, i.e., on the alkaline side of the isoelectric point, as it does in plasma. The change in pH due to the Donnan effect cannot be very great in a buffered solution such as plasma and is of about the same magnitude as the effect of escape of CO_2 so that the pH of the plasma in the sac must be practically the same after equilibrium is established as it is in the body. It can be stated with certainty that whatever change in pH takes place as the resultant of CO_2 escape and the Donnan effect is so slight that the error introduced in the osmotic pressure value is negligible. In brief, we may be sure that the osmotic pressure values observed represent the true osmotic pressure values exhibited by the plasma colloids in the intact animal.

For the first experiment a cannula was inserted into an aortic arch of a frog and about 6 cc. of blood drawn into a syringe containing 4 drops of 5 per cent potassium oxalate solution with pH of 7.2. The blood was transferred to a centrifuge tube and centrifuged under oil. The pH of the plasma was then determined by Cullen's method (12). The approximate isoelectric point of the proteins was determined as follows. Four drops of plasma were put into 5 cc. of potassium acid phthalate buffer solutions in each of 9 test tubes, the pH of the solutions ranging from 3.4 to 5 in steps of 0.2. These were boiled and allowed to stand one-half

hour. At that time precipitation of the proteins was most nearly complete, as evidenced by the least cloudiness, in the tube at pH 4.2. It is recognized that this method is not strictly quantitative but the isoelectric point so obtained is sufficiently accurate for our purposes. It would of course be absurd to speak of the exact isoelectric point of the plasma proteins since the point is somewhat different for the different proteins. It is sufficient for our purpose to show that the isoelectric point of the proteins is at such a distance from the pH of the plasma that the curve obtained by plotting the osmotic pressure as ordinates against pH as abscissae is nearly flat, as was shown by Loeb with albuminate solutions, in the region of the plasma pH.

The osmotic pressure was determined as follows. A collodion sac of about 1 cc. capacity was made to fit over a heavy-walled glass tube of 1 mm.

TABLE 1

PLASMA	APPROXIMATE ISOELECTRIC POINT OF PLASMA PROTEINS	OSMOTIC PRESSURE OF COLLOIDS	PER CENT PROTEIN IN PLASMA
pH	*pH*	*cm.* plasma	
7.65	4.2	9.6	2.40

TABLE 2

	OSMOTIC PRESSURE OF COLLOIDS	PERCENT PROTEIN IN PLASMA	OSMOTIC PRESSURE FOR EACH PER CENT PROTEIN IN PLASMA
	cm. plasma		*cm.* plasma
Frog 1	9.6	2.40	4.0
Frog 2	9.8	2.52	3.9
Frog 3	11.5	2.80	4.1

bore. The sac was almost filled with plasma and pushed on over one end of the tube, being pushed up until the plasma in the tube stood about 10 to 12 cc. above the sac. The sac was then tied on and dipped into a 250 cc. Erlenmeyer flask containing 0.7 per cent NaCl solution buffered and brought to a pH of 7.65 by adding Na_2HPO_4. Readings were then made at the end of eight hours. Correction was made for the rise of plasma in the tube due to capillary pressure. The protein content of the plasma was determined by the refractometer. The results are seen in table 1. In view of the fact that frog's urine is normally alkaline it is of interest to note that frog's plasma is considerably more alkaline than human plasma.

The osmotic pressure and protein content of the plasma were determined on two more frogs. The results, along with the osmotic pressure and protein figures of the first frog, are seen in table 2.

It is seen the osmotic pressure is so low that a glomerular capillary pressure of even 10 mm. Hg is amply sufficient to provide a pressure head for filtration.

DISCUSSION. In this paper we called attention to the fact that glomerular filtration in the mammalian kidney is not yet proved although the presumptive evidence in its favor is very strong. Indeed, until some method is devised for determining directly the glomerular capillary pressure in the mammalian kidney, it would seem that the task of establishing an absolute proof of glomerular filtration is well nigh impossible. The interpretation of the work presented in this paper is that the arguments of Hill and McQueen against glomerular filtration in the frog are not sound. We have shown that Hill and McQueen have not proved that glomerular filtration cannot occur; we have not proved that it does occur. Of course the demonstration of the fact that the pressure in the frog's glomerular capillaries exceeds the osmotic pressure exhibited by the plasma colloids is strong evidence in favor of the view that filtration does take place. The strict truth remains, however, that we have not yet proved that it does.

SUMMARY

The question of glomerular filtration is discussed. The osmotic pressure exerted by the colloids in the plasma of three frogs is shown to range from 9.6 to 11.5 cm. of plasma. It is shown that the colloidal osmotic pressure of frogs' plasma is of such a magnitude as to be exceeded by the pressure in the frog's glomerular capillaries.

The conclusion is drawn that the arguments of Hill and McQueen against glomerular filtration in the frog are not valid.

BIBLIOGRAPHY

(1) STARLING: Journ. Physiol., 1899, xxiv, 312.
(2) STARLING: Human physiology, 3rd ed., 1187.
(3) VOLKMANN: Die Hämodynamik. Leipzig, 1850, 195.
(4) ERLANGER AND HOOKER: Johns Hopkins Hosp. Repts., 1904, xii, 346.
(5) HOOKER: This Journal, 1910, xxvii, 24.
(6) GESELL: This Journal, 1913, xxxii, 70.
(7) GESELL: This Journal, 1914, xxxiv, 186.
(8) RICHARDS AND PLANT: This Journal, 1922, lix, 144, 184, 191.
(9) WOODLAND: Journ. and Proc. Asiatic Society of Bengal, 1922, xviii, 85.
(10) HILL AND MCQUEEN: Brit. Journ. Exper. Pathol., 1921, ii, 203.
(11) LOEB: Journ. Gen. Physiol., 1918–19, i, 39, 237, 363.
(12) CULLEN: Journ. Biol. Chem., 1922, lii, 501.

ABSORPTION OF INSULIN BY RECTUM

S. PESKIND, J. M. ROGOFF AND G. N. STEWART

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University

Received for publication February 22, 1924

So far as we know, no one has hitherto reported absorption of insulin when administered *per rectum* even in large doses. Banting and Best (1) state that rectal injections were not effective in reducing the blood sugar in a depancreatized dog. Woodyatt (2) tried this method of administration in patients, "with many variations," with a negative result. The question arises whether the insulin is destroyed in the lower bowel, or is simply not absorbed. The chances of escape from the destructive action of proteolytic ferments would seem to be better here than in the small intestine or stomach. Yet Hachen and Mills (3) and Fisher (4) have found that insulin placed in a loop of small intestine is absorbed rapidly. And Winter (5) has stated that this is also true when it is introduced into the stomach in 25 per cent alcohol, a result which he attributes to the fact that the solvent is itself known to be taken up easily by the gastric mucosa.

Experiments on rabbits. It was easily demonstrated that when insulin was injected into the rectum of rabbits in serum[1] or blood it was quickly absorbed, as shown by the characteristic effect upon the blood sugar. However, there is nothing specific in this, and the investigation, so far as it has gone, indicates that it is possible, in the rabbit at any rate, to alter the conditions in several ways so as to favor or to hinder the absorption of insulin. If success is ever to be attained in administering insulin for therapeutic purposes, by the alimentary canal, it must be by procuring favorable conditions for its absorption. In this paper it is intended to record some of the observations made, without attempting to analyze them or to offer explanations.

[1] The reason for testing the influence of serum was the suggestion (by S. P.) that the antitryptic action of serum might protect insulin administered *per rectum* sufficiently to permit it to be absorbed in effective amounts. It cannot be said that we have obtained any evidence that this is the explanation of the serum effect. While the research was under my general direction, and I was constantly in touch with it, the chief part in the actual work was taken by Doctor Rogoff and next to him by Doctor Peskind, acting under his guidance. G. N. S.

The experiments were made on animals which had fasted generally for 1 to 3 days, receiving water, however. Large doses of insulin were employed in many of the observations, as the object was to obtain a decided result. But it was shown that relatively small doses could also produce distinct effects, although they were usually high compared with the effective subcutaneous doses. In some of the experiments the liquids were introduced into the rectum through a tube inserted for 4 to 6 inches, in others by means of a syringe, the nozzle of which was about an inch within the anus, and in some by a syringe with a funnel-shaped opening just within the anus. No striking difference in the result could be associated with the manner of introduction. Sometimes the animal was held gently for 15 to 45 minutes after the injection, sometimes it was placed immediately in a cage. Occasionally a clip was put on the anus, but this is not necessary and was abandoned. Sometimes the clip seemed to increase the tendency to straining. If any material loss of injected liquid occurred this was noted and sometimes a second injection was made. It was not infrequently observed, however, that if the liquid was retained for a short time, subsequent loss of a portion of it did not noticeably affect the result. Serum and especially blood appear to be less easily retained by rabbits than water or salt solution. The dogs retained all the liquids injected more easily than rabbits. The blood sugar was estimated by the Folin-Wu method. Defibrinated blood was employed in the first few experiments, serum in the great majority. Although no strictly quantitative experiments were made it was shown sufficiently that the serum was the important thing. No clear difference in the effectiveness of serum from different animals (cat, ox, hog) was apparent. After standing several days in the ice chest a serum in some instances was proved to have become ineffective, but for the first few days no marked difference was observed. On account of the well known variability of the response of different rabbits to insulin a very large number of experiments would be necessary to establish such differences.

The effect of insulin in serum (or blood) was first compared with its effect when introduced in 0.9 per cent sodium chloride solution. The solutions were warmed to body temperature. The results of a number of the experiments with serum are assembled in table 1. Control experiments with insulin in 0.9 per cent sodium chloride solution are given in table 2. For a reason which will be apparent later a number of experiments, in which insulin was added to a 10 or 12 per cent solution of egg white in the 0.9 per cent salt solution, are included in table 2. In all the tables the blood sugar percentage before insulin administration is given in the column headed "initial." The percentages after insulin have attached to them in parentheses the number of minutes between the injection of the insulin and the drawing of the blood. The iletin

unit referred to is the new unit (Lilly). The numbers of the various specimens of iletin were of course always preserved in the records. By referring to the tables the citation of many protocols will be avoided. Occasionally, however, protocols will be given to bring out points which could not be included in the tables. Thus, in rabbit 916, in the first

TABLE 1

Rabbits. Insulin in serum

NUMBER OF ANIMAL	BODY WEIGHT	FASTED	INJECTED		BLOOD SUGAR (GRAMS IN 100 CC.)	
			Serum	Iletin	Initial	After injection (number of minutes in parentheses)
	k. m.	*days*	*cc.*	*units*		
874	2.27	1	20	10	0.097	0.080(19); 0.060(43); 0.078(81); 0.085(138); 0.091(195); 0.092(255)
875	1.67	1	5	2	0.105	0.127(22); 0.133(53); 0.121(100); 0.105(165); 0.118(245)
876	1.66	1	5	2	0.107	0.115(22); 0.114(49); 0.10(102); 0.095(192); 0.122(267)
880	1.01	2–2½	10	10	0.33	0.041(48); 0.036(95)
887	2.66	1	20	20	0.111	0.059(45); 0.048(75); 0.079(130)
889	2.15	1½	20?	20*	0.108	0.053(45); 0.08(115)
894	2.60	1	20	20	0.119	0.05(38); 0.081(108); 0.10(193); 0.111(253)
907	2.08	3½	20	25	0.09	0.061(38); 0.051(68); 0.038(113); 0.057(173)
913	1.86	3	25	0	0.093	0.110(43); 0.115(83); 0.111(120); 0.105(190)
914	2.43	3	20	25	0.093	0.057(35); 0.033(70); 0.053(110); 0.080(180)
916	2.00	2	38†	100†	0.141	0.053(50); 0.032(130); 0.035(230)
918	2	2	20	10	0.120	0.077(45); 0.067(100); 0.097(185); 0.102(305)
919			30	0	0.140	0.117(30); 0.120(60); 0.128(105); 0.129(150)
921	2	2	20	10	0.110	0.050(58); 0.047(110); 0.069(170); 0.088(250)
944	2		22	2	0.090	0.068(35); 0.056(60); 0.093(120)
947	1.5	2	5	20	0.128	0.069(50); 0.090(80); 0.120(120); 0.119(170)
948	1.5	2	10	20	0.106	0.056(35); 0.037(70); 0.044(100); 0.120(210)
952	2	2	15	20	0.106	0.046(55); 0.053(95)
955	1.5	2	15	8	0.096	0.056(20); 0.037(55)
956	1.25	2	15	4	0.094	0.042(32); 0.050(85)
957	2.31	2	15	2	0.095	0.057(30); 0.096(95)
963	2.25	2	120	5	0.095	0.126‡; 0.045(35); 0.027(100); 0.040(135)
964	1.64	2	60	5	0.134	0.163‡; 0.078(25); 0.062(70); 0.075(130)

* Lost at least half of it at intervals within ½ hour.

† In two equal instalments; much of first lot lost.

‡ After serum but before insulin.

In 916, 918, 919, 921 blood was used, in the other animals serum. In 876, 10 cc. of 0.9 per cent salt solution was added to the serum. In 963 the insulin was given in 5 cc. water after preliminary irrigation of the rectum with a large quantity of serum. In 955, 956, 957 and 964 the insulin was given with 5 cc. water 40 minutes after the serum. The first 14 experiments in this table were by J. M. R., the remaining 9 by S. P.

experiment made, an enormous dose was given purposely, and the animal eventually died in convulsions. It should be stated, however, that in the experiments with more moderate, although still large doses, convulsions, while sometimes seen, were rare compared with their frequency with subcutaneous administration of insulin.

Rabbit 916. Weight about 2 kgm. Fasted 52 hours. As always, water was allowed during the fasting period. Blood sugar (11:06 a.m.) 0.141 per cent. At 11:40 a.m. injected into rectum 20 cc. fresh defibrinated cat's blood with 50 units iletin. A good deal was lost. Blood sugar (12:30 p.m.) 0.053 per cent. At 12:40 p.m. injected 18 cc. of the cat's blood with 50 units iletin. At 1:45 p.m., convulsions. Blood sugar (1:50 p.m.) 0.032 per cent. At 3:30 p.m. slight convulsions. Blood sugar 0.035 per cent. At 3:37 the animal died in convulsions.

In the next pair of experiments, on rabbits 917 (table 2) and 918 (table 1), the effect of salt solution and serum was compared. The rabbits were of about the same weight (2 kgm.) and had fasted 2 days. But a much larger total dose of insulin was given with the salt solution. In spite of this there was little if any effect with the salt solution, while the serum caused a definite hypoglycemia. Several similar pairs of ex-

TABLE 2

Rabbits. Insulin in 0.9 per cent NaCl solution (with or without egg white)

NUMBER OF ANIMAL	BODY WEIGHT	FASTED	INJECTED		BLOOD SUGAR (GRAMS IN 100 CC.)	
			Solution	Iletin	Initial	After injection (number of minutes in parentheses)
	kgm.	*days*	*cc.*	*units*		
873	2.29	1	20	10	0.111	0.128(18); 0.112(55); 0.095(93); 0.095(155); 0.098(220); 0.090(280)
899	1.09	2-2½	20	20	0.113	0.112(28); 0.107(66); 0.102(118); 0.108(198)
900	1.33	2-2½	20	20	0.110	0.114(29); 0.10(67); 0.096(122); 0.105(197)
917	2.00	2	20	50	0.079	0.075(55); 0.077(125); 0.086(270)
920	2.00	2	20	50	0.113	0.144(55); 0.147(105); 0.126(165); 0.140(235)
969	2.39	2	20	20	0.09	0.11(40); 0.102(70); 0.10(110)
970	2.28	2	20	25	0.102	0.114(33); 0.095(68); 0.097(110)
972	1.44	2	20	20	0.099	0.095(33); 0.107(78); 0.098(118)
973	1.61	2	20	20	0.102	0.101(35); 0.108(75); 0.105(115)

In 969, 970, 972 and 973 the insulin was given in 20 cc. of a 10 per cent solution of egg white in 0.9 per cent NaCl, in the other animals in the salt solution without egg white. Experiments in this table by J. M. R.

periments were made to compare the effect of salt solution and blood or serum, and always with the same result. Occasionally there might even seem to be a small increase in the percentage of blood sugar after insulin was injected in salt solution, as in rabbit 920 (table 2). The companion experiment with blood, rabbit 921 (table 1), showed a marked hypoglycemia, although the dose of insulin was only one-fifth as great.

An experiment on rabbit 919 (table 1) showed that fresh defibrinated cat's blood did not itself cause the hypoglycemia observed with insulin in blood. In other experiments this was shown definitely for serum. It is impossible to attach any significance to the apparent slight decline in the sugar percentage after the injection of blood into the rectum in rabbit 919. Never has anything which could properly be called a hypoglycemia been seen with blood or serum alone. Sometimes a certain,

even a considerable degree of hyperglycemia may be observed. For example, in rabbit 913 (table 1) the blood sugar remained almost steady after injection of serum, whereas in rabbit 914 administration of the same amount of the same serum with insulin caused a drop in the sugar to 0.033 per cent. In rabbit 946 fresh cow's serum alone caused an apparent increase in the blood sugar percentage, whereas administration of the same serum (24 hours later) with 10 units of insulin to rabbit 874 (table 1) and (48 hours later) in much smaller amount with 20 units of insulin to rabbit 947 (table 1) was followed by a distinct hypoglycemia.

A few experiments were made with different volumes of serum and the same dose of insulin to see whether the quantity of serum was a significant factor. In view of the unavoidable variability of the material little can be said, except that 5 cc. of serum seemed generally to be less effective than 10 cc., while 10 cc. seemed, sometimes at any rate, to be as effective as larger quantities. Some observations illustrating this point will be found in table 1.

No attempt was made to determine the minimum dose of insulin which would produce a definite effect upon the blood sugar when introduced with serum *per rectum*. It was seen, however, that 5, 3 and, in one or two instances, even 2 units were effective when administered with an appropriate volume of serum (see table 1, experiments 944, 956, 957, 963, 964). The negative result of 2 units in rabbits 875 and 876 (table 1) may have been due to an inadequate quantity of serum (5 cc.). But in 876 10 cc. of 0.9 per cent sodium chloride solution was added to the serum, and insulin in salt solution is ineffective (table 2). The serum in 876 was fresh beef serum. In 875 it was cow's serum 2 days old, but which was shown to be effective on the same day in the same amount with 20 units of insulin, in rabbit 947 (table 1).

In one or two animals the effect of a preliminary irrigation of the rectum with serum was tried. It was supposed that in this way the action of the serum, whatever it is, might be exerted upon the mucosa while at the same time the bowel would be cleared of feces. The experiment on rabbit 963 (table 1) is an example. The administration of 5 units of insulin caused a marked effect. It was not known at this time that insulin in water is absorbed in effective amount when introduced into the rectum, and it cannot be definitely stated what, if any, part the small quantity of water given with the insulin may have taken in the reaction.

Rabbit 963 (table 1), 2.25 kgm. Fasted 2 days. Blood sugar (4:55 p.m.) 0.095 At 5:00 p.m. to 5:15 injected slowly through funnel and catheter 70 cc. calf's serum (2 days old); much of it escaped. At 5:15 p.m. injected a little more, which was not retained. At 5:30 p.m continued injection, making the total amount 120 cc Blood sugar (5:40 p.m.) 0.126. At 6:00 p.m. injected 5 units iletin with 5 cc. water. Blood sugar (6:35 p.m.) 0.045; (7:40 p.m.) 0.027 per cent. At 7:45 p.m. a convulsion, and again at 8:00 p.m. Blood sugar at 8:15 p.m., 0.040 per cent. At 9:00 p.m. the animal was quiet and rather dull, but recovered.

In another rabbit, 955 (table 1), the initial blood sugar was 0.096 per cent. Calf's serum (15 cc.), obtained 2 days previously, was injected and 40 minutes thereafter 8 units insulin in 4 cc. water. Twenty minutes later, the blood sugar was 0.056, and after 35 minutes more 0.037 per cent. The animal now developed convulsions, but recovered.

The experiment was repeated next day on another rabbit, 956 (table 1). The same serum (now 3 days old) was injected (15 cc.), and 35 minutes thereafter 4 units insulin with 5 cc. water. The blood sugar was reduced from 0.094 to 0.042 (32 minutes after insulin) and was 0.050 per cent, 85 minutes after insulin. In another rabbit, 957 (table 1), 15 cc. of the same serum (3 days old), and then, after 40 minutes, 2 units of insulin with 5 cc. of water were introduced. The blood sugar fell from 0.095 before the injection to 0.057 in 30 minutes, returning (95 minutes after the insulin injection) to 0.096 per cent. Three days later, the serum being now 6 days old (having been kept in the ice chest), a similar experiment was made on two rabbits (960 and 961), each weighing 2.3 kgm., and kept fasting for 2 days. Five units of insulin were injected in each case, but no diminution in the blood sugar was caused. Apparently, then, the favoring effect of the serum on insulin absorption was lost between the end of the 3rd and the 6th day.

In another serum (cow's) a similar phenomenon was observed. Two days after it was obtained a marked effect was caused by the injection of 20 units insulin in 10 cc. of serum into the rectum of rabbit 948 (table 1). The blood sugar fell from 0.106 to 0.056, 35 minutes after the injection; 0.037, 35 minutes later; 0.044, 100 minutes after the injection; returning to 0.120 per cent $3\frac{1}{2}$ hours after injection. The symptoms disappeared and the animal recovered completely. On the next day, the serum being now 3 days old, no effect was caused by 15 units of insulin in 20 cc. of the serum in another rabbit (878), weighing 2.32 kgm. and fasting for 36 hours. The negative result was confirmed on another rabbit on the next day, the serum being then 4 days old. It would therefore seem that this serum had lost its favoring action between the end of the 2nd and the 3rd day. However, it is not uncommon to see the action persist for 4, 5 or more days, the serum being kept on ice. Thus, rabbit 925, weighing $1\frac{1}{2}$ kgm., received 20 cc. of calf's serum, 78 hours old, with 20 units of insulin. The blood sugar fell from 0.143 to 0.044 per cent in 1 hour. When quite fresh, 20 cc. of the serum with 10 units insulin caused in a $1\frac{1}{2}$ kgm. rabbit (923) a reduction in the blood sugar from 0.116 to 0.041 per cent in 75 minutes, although a considerable amount of the liquid was lost.

Heating serum for 35 minutes at 60° to 62°C. was found to abolish the favoring action on the absorption of insulin. In one experiment it was first shown that fresh hog's serum (10 cc.) with 10 units iletin injected

into the rectum of rabbit 880 (table 1) reduced the blood sugar content from 0.133 to 0.041 in 48 minutes, and to 0.036 per cent in 47 minutes more. Dextrose was injected subcutaneously and the animal recovered completely. A similar experiment was made on rabbit 879, whose weight was the same as that of rabbit 880, 1.03 kgm. (after fasting 36 hours). But instead of serum, 10 cc. of sediment of the same blood, still containing a fair proportion of serum, was injected and 10 units of iletin. The blood sugar percentage was reduced from 0.125 to 0.08 in 49 minutes, and was 0.133 in 55 minutes more. Some of the serum was now heated for 35 minutes at 60° to 62°C., and 10 cc. of it with 10 units iletin introduced into the rectum of rabbit 881, weighing 0.93 kgm. which had fasted 48 to 60 hours. The blood sugar was unaffected, being 0.125 before the injection; 0.128, 45 minutes thereafter; and 0.120 per cent after an hour more.

One experiment was made to determine whether the favoring action would still be obtained from either the globulin or the albumin fraction. The globulin was precipitated by half saturating with ammonium sulphate, and the albumin by saturating the filtrate (6). The two fractions were dialysed in running water, but no attempt was made to purify them, or to get rid of all the salt. The globulin from 15 cc. of serum, with 20 units iletin, was injected into rabbit 885, weighing 2.98 kgm., after fasting 3 days. No significant change was produced in the blood sugar. About half of the albumin fraction from 15 cc. of serum, with 20 units iletin, was injected into rabbit 953, weighing 2 kgm. after fasting 2 days. No hypoglycemia was caused. The original serum was still effective, since 15 cc. of it with 20 units iletin, injected into rabbit 952 (table 1), reduced the blood sugar from 0.106 to 0.046 per cent in 55 minutes.

In another experiment cow's serum was heated at 68° to 63°C. for 40 minutes, which precipitated the proteins in gelatinous form. Administered to the amount of 20 cc., with 15 units of iletin, it caused no change in the blood sugar of rabbit 877, weighing 2.41 kgm. The serum, however, was 3 days old, and although it had been quite effective on the previous day, the unheated serum now gave a negative result in rabbit 878 with 15 units of iletin.

The facts described indicated that the effect of serum might be due to some specific property or substance, and so far nothing had been made out, with the exception perhaps of the rough experiment on the protein fractions, which was inconsistent with the idea that an antitryptic action might be a factor. This was rendered more plausible when it was found that watery solutions of egg white facilitated the insulin action, when given *per rectum*, much as serum did. For Vernon (7) states that egg albumin has a powerful antitryptic effect.

In rabbit 891 (table 3) 20 cc. of a 12 per cent solution of egg white in tap water, with 20 units of iletin, caused a reduction in the blood sugar from 0.149 to 0.076 per cent in 38 minutes. In another rabbit, 883 (table 3), 20 cc. of a 10 per cent solution of egg white in tap water with 10 units of iletin produced a fall of the blood sugar percentage from 0.093 to 0.055 in 40 minutes.

However, it was soon found that this apparent favoring effect of egg white could not well be due to an antitryptic action. For first, it was not obtained when insulin stirred up with undiluted egg white was introduced into the rectum. In the experiment on rabbit 884, for example, the effect was, if anything, rather a moderate increase than a decline in the blood sugar percentage, which was 0.105 before the injection; 0.148, 39 minutes after it; 0.141 in 41 minutes more; and 0.097 in the last observation (148 minutes after the injection). Egg yolk gave no definite effect (rabbits 931, 932). Secondly, when egg white, diluted 10 times with 0.9 per cent sodium chloride solution instead of water, was injected with insulin the blood sugar content was not affected (see table 2, rabbits 969, 970), just as is the case with insulin in salt solution alone (table 2).

Since in rabbits insulin in water alone is absorbed from the rectum so as to produce a marked hypoglycemia, there is at present no reason to attribute the apparent effect of egg white solutions in water to anything else than the water. This is the reason for the inclusion of the observations on egg white in salt solution and in water respectively in tables 2 and 3. If anything, the addition of the egg white to water seemed to render the hypoglycemia less marked, and particularly less prolonged, than if water alone had been given with the insulin. But in view of the variability of the effect in different rabbits the number of experiments is too small to permit any definite conclusion to be drawn in regard to this point. An example of the blood sugar changes following injection of 20 units iletin in distilled water is seen in rabbit 897 (table 3). The sugar percentage fell from 0.095 to 0.039 in 33 minutes. It had not risen at all from this low level 71 minutes after the injection. In another hour it was 0.077, and in $3\frac{1}{2}$ hours after the injection it had returned to 0.087. In another somewhat larger rabbit, 898 (table 3), the same dose caused a smaller but quite distinct hypoglycemia. The same dose of iletin in a corresponding volume of 0.9 per cent salt solution had no effect on the blood sugar of two rabbits (899 and 900, table 2) of similar size, which had fasted even slightly longer. The sample of iletin was always the same in experiments which are compared. In two other (larger) rabbits (908, 909, table 3) which had fasted 108 hours, 25 units of another specimen of iletin in 20 cc. of water were administered. The blood sugar fell from 0.093 to 0.042 per cent in one of the animals, and from 0.114 to 0.058 per cent in the other. A control experiment with

25 cc. of distilled water alone in two rabbits (910, 911, table 3) showed that the blood sugar was unaffected.

It has already been stated that while convulsions associated with a marked hypoglycemia have been seen in rabbits after rectal administration of insulin in serum, they are apparently not so common as when a similar reduction of the blood sugar is caused by hypodermic injection of insulin to or below the "critical" level of Macleod and his co-workers. We forbear to speculate on the reasons for this. One of them is possibly the fact that although the blood sugar percentage with the large doses

TABLE 3

Rabbits. Insulin in water (with and without egg white)

NUMBER OF ANIMAL	BODY WEIGHT	FASTED	INJECTED		BLOOD SUGAR (GRAMS IN 100 CC.)	
			Solution	Iletin	Initial	After injection (number of minutes in parentheses)
	kgm.	*days*	*cc.*	*units*		
882	1.5	2	20	20	0.091	0.061 (42); 0.090 (87); 0.088 (148)
883	1.45	2	20	10	0.093	0.055 (40); 0.083 (83); 0.091 (146)
891	2.55	1½	20	20	0.149	0.076 (38); 0.112 (98); 0.133 (188)
896	2.52	¾	20	20	0.111	0.075 (70); 0.109 (175); 0.109 (285)
897	1.16	1½–2	20	20	0.095	0.039 (33); 0.040 (71); 0.077 (127); 0.087 (208)
898	1.41	1½–2	20	20	0.103	0.067 (42); 0.084 (72); 0.083 (130); 0.091 (218)
902	2.25	1½–2	10	12	0.10	0.045 (30); 0.057 (65); 0.077 (115); 0.105 (180)
904	1.53	2½	10	5	0.091	0.083 (32); 0.083 (70); 0.095 (117)
905		3½	12	20	0.080	0.044 (45); 0.057 (90)
908	2.37	4½	20	25	0.093	0.042 (30); 0.053 (75); 0.073 (140); 0.08 (200)
909	2.41	4½	20	25	0.114	0.071 (32); 0.058 (77); 0.07 (134); 0.082 (197)
910	1.63	2	25	0	0.091	0.091 (30); 0.095 (58); 0.102 (93)
911	2.44	2	25	0	0.111	0.10 (32); 0.103 (63); 0.099 (101)
974	1.65	2	20	20	0.105	0.63 (35); 0.091 (75); 0.11 (110)

In 882, 883, 891 and 974 the insulin was given in a 10 per cent solution of egg white, in 896 in a 14 per cent solution, in the other animals in water without egg white; 905 was the same rabbit as 904 again used after fasting another day. Experiments in this table by J. M. R.

of insulin employed by us is rapidly reduced, the hypoglycemia is perhaps not so durable with this mode of administration. This is true also of the hypoglycemia caused by insulin in water. Although the minimum reached may be as low as with serum, we have not hitherto observed convulsions when the insulin was administered in water. That the convulsions when associated with a low blood sugar content in the serum-insulin experiments were due to the insulin does not seem doubtful, and in one or two instances they were shown to be relieved by administration of dextrose. But it is advisable to avoid dogmatism on the question whether they are always entirely due to the insulin, as is illustrated by

an experiment on two rabbits. Rabbit 906 weighed 1.13 kgm. and rabbit 907, 2.08 kgm. They had been caused to fast 84 hours with the idea of favoring the onset of insulin convulsions. Each received 25 units of iletin in 20 cc. of fresh beef serum. In 907 the blood sugar fell from 0.09 to 0.061 per cent in 38 minutes, then to 0.051 in 30 minutes more; to 0.038 in another 45 minutes, and was 0.057 per cent 3 hours after the injection. There were no convulsions, and the animal recovered perfectly. The course of events in rabbit 906 is best shown by a short protocol.

Rabbit 906. At 10:40 a.m. blood sugar 0.118 per cent. At 10:52 a.m. insulin in serum injected. Blood sugar (11:30 a.m.) 0.151; (12:00 m.) 0.143 per cent. The animal is getting feeble. At 12:45 p.m. blood sugar 0.115 per cent. The hind end seems paralyzed. At 1:50 p.m. blood sugar, 0.099 per cent. Hind end paralyzed. On being stirred up the rabbit made 2 or 3 small convulsive movements with the fore paws, then seemed exhausted. At 2:25 p.m. seemed to be dying, and at 2:48 there was a convulsion and the animal died. The abdominal and pleural cavities contained a considerable quantity of fluid.

This was the only rabbit, out of considerably more than 40 which received serum and insulin, in which such a combination of symptoms was seen. The question may be asked whether the animal was poisoned by the serum, perhaps because of some digestive disturbance. It is impossible to say. The stomach contained a considerable amount of food in spite of the period of fasting.

Experiments on dogs. The results on the rabbit were so constant that we were surprised to obtain no effect upon the blood sugar in dogs, whether insulin was administered in serum, or in water. In check experiments on rabbits insulin, in the same or even smaller doses per kgm. of body-weight and in the same proportional volume of serum or water, gave the usual effects. Relatively large doses of insulin were generally used so as to ensure a good effect in the rabbits. The same lots of (hog or ox) serum and of iletin were, of course, employed for the dog and the control rabbit. The results are given in the tables—rabbits 887 and 889 (table 1) and dogs 886, 888 and 890 (table 4) with serum; rabbit 904 (table 3) and dog 903 (table 4) with water. Rabbit 889 lost a considerable portion of the iletin-serum administered, but nevertheless developed a marked hypoglycemia. In rabbit 904, after a fast of 60 hours, 5 units of iletin in 10 cc. of water caused no definite fall in blood sugar. It was left without food for 20 hours more and then given 20 units of iletin in 12 cc. of water (experiment 905, table 3). The blood sugar fell from 0.08 to 0.044 per cent in 45 minutes, and was 0.057 per cent 45 minutes later.

In one dog (892, table 4), weighing 9.7 kgm. after a fast of 36 hours, 100 units of iletin in 100 cc. of a 12 per cent solution of egg white in water produced no effect upon the blood sugar.

A similar experiment (895, table 4) with egg white was performed on a dog 7 days after complete pancreatectomy, with no decided effect upon the hyperglycemia. In the check experiment on the rabbit (896, table 3) the reduction of the blood sugar was distinct (from 0.111 to 0.075 per cent in 70 minutes), although not so great as usual. The animal had fasted for only 18 hours. Also the interval between injection of the iletin and withdrawal of the next blood specimen was longer than usual, and a smaller sugar percentage might have been seen had it been taken earlier. Two days after the removal of the pancreas (experiment 893, table 4) 10 units of iletin per kgm., in 10 cc. of fresh ox serum per kgm., were given, with little if any reduction of the blood sugar.

TABLE 4

Dogs

NUMBER OF ANIMAL	BODY WEIGHT	FASTED	INJECTED		BLOOD SUGAR (GRAMS IN 100 CC.)	
			Liquid	Iletin	Initial	After injection (number of minutes in parentheses)
	kgm.	*days*	*cc.*	*units*		
886	7.0	2	70*	75	0.097	0.095 (60); 0.083 (120)
888	6.2	1	60*	60	0.108	0.097 (48); 0.10 (150)
890	7.7	1½	70*	75	0.091	0.102 (45); 0.103 (105); 0.086 (180); 0.095 (255)
892	9.7	1½	100†	100	0.093	0.095 (45); 0.090 (105); 0.080 (200); 0.085 (295)
893	6		60*	60	0.282	0.266 (45); 0.250 (120); 0.263 (200); 0.241 (265)
895	5.6		20†	75	0.174	0.176 (60); 0.154 (165); 0.166 (275)
901	5.5		45‡	45	0.254	0.267 (57); 0.263 (97); 0.235 (172)
903	6.5	2½	65‡	75	0.087	0.081 (35); 0.085 (70); 0.086 (120)

* Serum.

† Egg white (12 per cent solution in water).

‡ Water.

Experiments 893, 895 and 901 were done on the same depancreatized dog. Experiments in this table by J. M. R.

A somewhat smaller proportional dose (8 units per kgm.) in a rabbit (894), which had fasted 24 hours, elicited the usual hypoglycemia, the blood sugar falling from 0.119 to 0.05 per cent in 38 minutes.

Iletin in distilled water caused no effect in the depancreatized dog 13 days after the operation (experiment 901, table 4). The ordinary hypoglycemia was produced in a control rabbit (902, table 3), the blood sugar falling from 0.10 to 0.045 per cent in 30 minutes. It was 0.057 per cent 35 minutes later, and 0.077 per cent after another 50 minutes. An hour later it had returned to the initial value.

As already stated, we do not consider it advisable at present to discuss the explanation of the different results obtained with the various menstrua employed as regards the absorption of insulin, or the difference between

the rabbit and the dog. It is, however, instructive that so apparently small a difference as that between water and physiological salt solution, or as that between heated and unheated serum should determine whether insulin (in the rabbit) should be relatively well absorbed or not absorbed at all, or at least not absorbed in the amount and at the rate required to affect the blood sugar content. These things at least suggest that by altering the conditions, perhaps in quite simple ways, absorption of this substance from the alimentary canal or from particular parts of it may be greatly facilitated. It is possible that water being taken up more rapidly than the salt solution from the lower bowel, carries the insulin with it in sufficient quantity to produce its effects, and that the same is true of one or more of the serum proteins, which might be expected to lose this property when their state of aggregation was altered by heating. As to the difference between the rabbit and dog one might think of serum as being more foreign to the rabbit's bowel than to that of the carnivorous animal, and as therefore capable, perhaps, of causing some injury, physiological if not anatomical, which might permit the passage of insulin. But it is against this idea that the difference between the two animals is just as marked in the case of water. In any case it seems probable that we failed to obtain insulin effects by rectal administration in the dog, while obtaining them easily in the rabbit, because we do not yet know how to overcome the obstacles in the dog, rather than because these obstacles are insuperable. Whether the differences between the two animals are connected with differences in the feces as regards their physical or chemical properties, reaction, etc. cannot be profitably discussed at present.

SUMMARY

Insulin injected *per rectum* into rabbits along with blood, serum or water is absorbed and elicits the characteristic effect on the blood sugar content. The effect is rapidly produced, although it may not be as durable as with subcutaneous injection. Larger doses are also necessary as a rule. Injected in 0.9 per cent sodium chloride solution, it does not cause any significant change in the blood sugar, and especially no hypoglycemia. The menstrua which are suitable for favoring the taking up of insulin by this method of administration in the rabbit do not appear to be suitable for the dog, since negative results were obtained with the liquids mentioned both in normal dogs and in a depancreatized animal.

BIBLIOGRAPHY

(1) Banting and Best: Journ. Lab. Clin. Med., 1923, vii, 251.
(2) Woodyatt: Journ. Metab. Res., 1922, ii, 793.
(3) Hachen and Mills: This Journal, 1923, lxv, 395.
(4) Fisher: This Journal, 1923, lxvii, 65.
(5) Winter: Journ. Physiol., 1923, lviii, 18.
(6) Cathcart: Journ. Physiol., 1904, xxxi, 497.
(7) Vernon: Journ. Physiol., 1904, xxxi, 346.

THE EFFECTS OF INTRAVENOUS INJECTIONS OF GLUCOSE AT A CONSTANT RATE ON BLOOD SUGAR AND HEMOGLOBIN CONCENTRATIONS[1]

E. C. ALBRITTON

From the Laboratories of Physiology, Ohio State University

Received for publication February 27, 1924

Little direct study has been made, apparently, of the rate of sugar intake into the blood as a factor in the course of the blood sugar curve after glucose administration. Observations during continuous intravenous injection of glucose have been reported by Woodyatt (1), and by Macleod and Fulk (2). Woodyatt apparently studied one normal unanesthetized dog. The dogs used by Macleod and his associates were anesthetized and were subjected to laparotomy and to accompanying injections of acid or alkali. Both investigators found that the blood sugar rose, and remained elevated as long as the injection continued. A similarly prolonged elevation of the blood sugar was observed by Allen and Wishart (3) in normal dogs, during the course of frequently repeated intravenous injections of glucose. The finding of Woodyatt and his coworkers (4) that the kidney threshold for sugar is passed when the rate of intravenous injection exceeds 0.85 gram of glucose per kilo per hour would seem to indicate that the height of the elevation varies with the rate of intake of sugar into the blood. That it is also influenced by the volume of the liquid injected with the sugar has been shown more recently by the same investigator (1).

Although the actual rate of intake into the blood after alimentary or subcutaneous administration cannot be precisely determined, a comparison of the blood sugar curves after different quantities of glucose administered by these routes contributes evidence on this problem.

The height of the curve has been shown by several investigators to be influenced by the size of the dose. Eadie (5) has found that of two different quantities injected subcutaneously in rabbits, the larger is followed by a higher elevation. The height of the rise after doses by mouth in man has been found by Staub (6) to vary with the size of the dose for quantities up to 80 grams. Maclean and DeWesselow (7) place this limit at 25 grams. Apparently a limit to the rate of alimentary ab-

[1] The work herein reported was carried out during the incumbency of a Medical Fellowship assigned by the National Research Council, Washington, D. C.

sorption prevents larger doses from causing still higher elevations of the blood sugar.

The duration of the elevation has also been shown to vary with the size of the dose. Staub (6), Maclean and DeWesselow (7) and Hansen (8) have observed that after larger doses of glucose in man hyperglycemia persists longer than after smaller doses. Staub used single doses varying from 10 to 80 grams. He also reported one curve in which the blood sugar was kept above fasting level for five hours by repeated small doses. Maclean and DeWesselow gave from one to three doses of 50 grams at 15 minute intervals. Hansen gave as high as 200 grams in a single dose. This quantity was followed by a much more prolonged hyperglycemia than were the more usual smaller doses. Eadie (5) observed longer curves after the larger doses given subcutaneously to rabbits than after the smaller. Boe (9) gave rabbits by mouth 1 gram per hour for several hours. In one experiment the blood sugar was thus kept elevated for 10 hours. He also found that single doses by mouth in 10 per cent solution caused a higher rise of blood sugar than the same doses in more dilute solution.

Boe and Staub have shown, however, that the curve gradually falls to fasting level even during the administration of repeated doses (6), (9). In a number of experiments by other observers, two doses were given, separated by an interval of one or one and a half hours, during which time the blood sugar had returned to fasting level. In these cases either no rise occurred after the second dose or the rise was significantly lower than after the first [Bang (10); Traugott (11); Foster (12)]. Bang worked with rabbits, the others with men. No significant lowering of the second curve was noted, on the other hand, by Maclean and DeWesselow (7) in the one experiment they reported. In a number of similar experiments by Meyer-Börnicke (13), in which, however, the blood sugar had not returned to fasting level in the interval, it either remained elevated or rose further after the second dose. The total duration of the elevation was approximately 2 hours from the first dose. Most of the curves were from patients, none of them diabetic. In experiments in which a glucose tolerance test was made 3 hours after a small carbohydrate breakfast Killian (14) reported blood sugar curves in normal persons that were not essentially different from the curves ordinarily obtained without preliminary feeding.

The trend of the foregoing evidence indicates that subsequent doses of glucose may not be so effective as the first dose in causing elevation of blood sugar. In ordinary alimentary blood sugar curves absorption is still in progress when the fall begins, as shown by Beeler, Bryan, Cathcart and Fitz (15). Whether the fall is due to a slowing of the rate of absorption or to the entrance into the situation of some factor which facilitates

the disappearance of sugar from the blood has not been determined. The latter has been the usual assumption, notably in the paper of Maclean and DeWesselow (7). These observers suggest that there is a threshold of glycogen formation which is reached when the blood sugar rises to near the kidney threshold, and that the fall of the alimentary blood sugar curve after the apex is passed is due to the sudden initiation of glycogen formation at this level.

The work herein reported was planned to throw more light, if possible, upon the rôle of the rates of intake and of disappearance of sugar in determining the course of the blood sugar curve.

METHOD. Glucose in 20 per cent solution was injected intravenously in dogs at the rate of 0.7 gram of glucose per kilo per hour for a period usually of 2½ hours. Blood samples were drawn at frequent intervals and the sugar and hemoglobin concentrations determined. Twenty-five experiments were made upon 13 dogs.

A 21 per cent solution by weight of "Baker's Analyzed" glucose was made with distilled water, divided into 100 cc. portions in small flasks, and autoclaved for 10 to 15 minutes at 5 to 10 pounds pressure. Several titrations with Benedict's quantitative copper solution showed a diminution of the reducing power to that of glucose of approximately 20 per cent. The solution was prepared in 3 or 4 liter quantities. Thus any slight error in percentage was made constant over a large number of experiments.

The dogs were apparently in good health, except for beginning mange in a few and a possible cystitis in no. 21. They were trained for the experiments for variable periods from 1 day to 2 weeks. Training consisted of frequently repeated periods of enforced lying upon a cushioned table. The dog lay upon his side, with feet and head loosely fastened to the table. Sometimes the training included accustoming the dog to lie without effort to rise even during hammering on the table underneath his head or other noisy procedures. Length of training depended upon the quickness with which the animal learned, but was influenced in some cases also by scarcity of animals.

During the experiment, in most cases, there was at some time evidence of restlessness. This showed itself in movements of the feet or head or sometimes in gentle whining. Petting or the command to "lie still" was usually sufficient to restore quiescence. Most of the time on the table was spent in simply lying quietly or in dozing.

The injection was made from a burette by way of rubber tubing through a Luer needle into the saphenous vein. The rate of flow was controlled by an assistant whose attention was given solely to this task. He was provided with a schedule showing the position of the meniscus at the end of each minute, and with a watch hung in convenient position for constant reference. Our later experience with the Woodyatt pump has

shown that without a schedule, and frequent change of adjustment during an experiment, an equal degree of accuracy, i.e., correction to the minute, cannot be expected. This is due to the sensitiveness of the pump to fluctuations in the electric current.

Blood was drawn from the external jugular vein through a Luer needle which was kept in place throughout the experiment. It was kept patent by a very slow flow of 0.9 per cent sodium chloride solution into it from another burette. The amount of fluid so introduced was usually relatively slight, but is taken into account in evaluating the data secured. When a sample was to be drawn the tubing leading from the burette was slipped off the needle and suction was made with a Luer syringe until blood appeared. After thus rinsing the salt solution out of the needle another dry, vaselined syringe was substituted and the sample taken. Blood was transferred to a test tube containing 5 to 10 mgm. of powdered potassium oxalate. Three cubic centimeters were usually taken at a time. The samples were taken before the injection began, and at approximately 20, 30, 40, 50, 63, 75, 110 and 150 minutes thereafter.

The Myers and Bailey (16) modification of the Lewis Benedict method of blood sugar analysis was used. A 2 cc. sample was utilized. A uniform quantity of picric acid crystals was added at once, the test tube stoppered and thoroughly shaken, after which the contents were poured directly on the filter without centrifuging. Two 3 cc. portions of the filtrate were separately carried through the remaining procedures, thus checking all the meaurements except that of the blood (2 cc.) and the water used for its dilution (8 cc.). The duplicates usually differed from their mean by no more than 5 mgm. per 100 cc., although sometimes wider differences were encountered.

In a number of the experiments a fasting sample was taken from the saphenous vein as well as from the jugular. This sample was taken with the hope that the difference in fasting blood sugar levels in the two veins might be correlated with length of fast or height of curve, but no correlation was found. The fasting samples were usually taken not longer than 15 or 20 minutes before the injection began.

In all but the first few experiments duplicate hemoglobin determinations were made on every blood sample. They are presented in table 1 in connection with the corresponding blood sugar values as evidence of the degree of dilution of the blood. In experiments 9, 10, 11 and 12 the Dare instrument was used. In the remaining experiments the Palmer method was used (17), taking a preparation of the fasting blood as a standard. In most of the experiments this fasting sample was read also in the Dare instrument and the hemoglobin values thereafter calculated to correspond to this value. The duplicates usually differed from their mean by no more than 1 or 2 per cent.

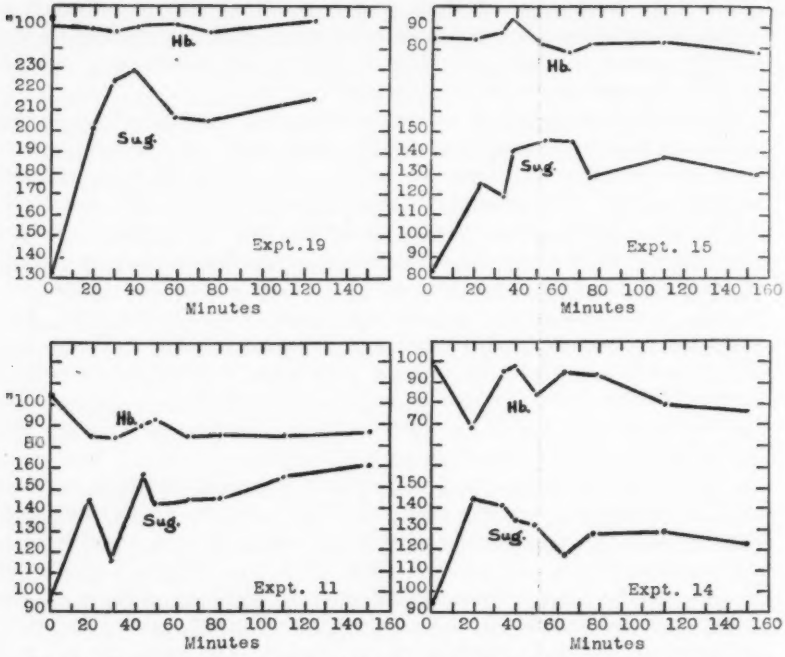


Fig. 1. Representative curves of blood sugar and hemoglobin concentrations during intravenous injection of glucose, 0.7 gram per kilo per hour. From data in table 1.

Observations. Data of the experiments are presented in protocols, in tables 1 and 2, and in figures 1 and 2. In table 1 the blood sugar and hemoglobin values are grouped within 10 minute periods in columns 6 to 11. Column 11 includes all samples taken between 65 and 85 minutes; column 12, those taken from 85 to 125 minutes; and column 13, samples taken between 125 and 165 minutes. At the head of each column is the mean time of all the samples with the standard deviation following. In a few experiments more than one sample was taken within the period of 85 to 125 and of 125 to 165 minutes. The values of these samples and the times at which they were taken have been averaged and are recorded as though only one sample of the average value had been taken.

In table 2 all the observations on blood sugar changes have been summarized as the average *elevation above fasting level* at the different time points indicated. Thus at 20 ± 1 minutes after the injection began, in 15 experiments on 12 dogs the blood was found to average 50 ± 16 mgm. per 100 cc. above the initial level. This average has been calculated by

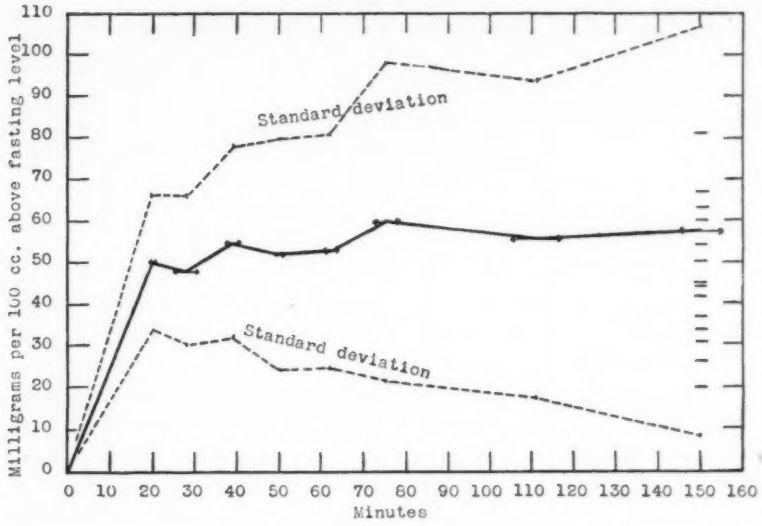


Fig. 2. Average elevation of blood sugar above fasting level during continuous intravenous injection of glucose, 0.7 gram per kilo per hour. From data in table 2. The elevation of the component curves at 150 minutes indicated. Three curves end at levels outside the limits of the figure, at 212, 137, and −12 mgm. per 100 cc. above fasting level.

subtracting each fasting jugular blood sugar value in column 4, table 1, from the 20 minute value for the same experiment in column 6, and averaging the differences.

This method has been chosen as defining more precisely the course of the first portion of the component blood sugar curves than would an average of the absolute value of the blood sugar at each time point. Obviously this is due to the elimination of the standard deviation of the fasting samples. As may be readily seen by averaging the values as they stand in each column in table 1, the two methods of presentation give average curves that run practically an identical course, except that the range of the expected variation above and below this average is narrower in the first portion of the curve as here presented (curve in fig. 2 plotted from data in table 2).

Discussion. These experiments have yielded blood sugar curves that, when averaged (fig. 2), form a level plateau in the period in which the ordinary alimentary blood sugar curve is falling. The elevation of the plateau averages 50 mgm. per 100 cc. above the fasting level. This

TABLE 1

Blood sugar, milligrams per 100 cc., and hemoglobin per cent

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
EXPERIMENT	DOG	ITEM	INITIAL VALUE		15-25 (20 ± 1) MINUTES	25-35 (28 ± 3) MINUTES	35-45 (39 ± 2) MINUTES	45-55 (50 ± 1) MINUTES	55-65 (62 ± 2) MINUTES	65-85 (75 ± 3) MINUTES	85-125 (111 ± 6) MINUTES	125-165 (150 ± 5) MINUTES
			Jugular	Saphenous								
1	13	Minute				30	44		60			
		Sugar	70			113	140		126			
		Hemoglobin										
2	15	Minute				25	38	51		71	119	150
		Sugar	104	100		170	180	212		215	177	185
		Hemoglobin										
3	21	Minute				25	37			75	115	150
		Sugar	95	88		133	143			124	115	149
		Hemoglobin										
4	13	Minute				25	37	50	63		90	137*
		Sugar	89	75		139	129	117	113		100	126*
		Hemoglobin										
5	21	Minute				25	37	50	63		105†	140‡
		Sugar	112	109		132	146	145	146		138†	146‡
		Hemoglobin										
6	21	Minute				25	37	53		75	110	150
		Sugar	98	95		123	171	185		268	265	310
		Hemoglobin										
7	15	Minute				25	39	50	63	78	113	157
		Sugar	104	97		147	115	104	101	93	113	92
		Hemoglobin										
8	23	Minute				25	37	50	63	75	109§	156
		Sugar	96	116		170	159	168	178	165	150§	146
		Hemoglobin										
9	24	Minute			19	29	39	49		76¶	109	149
		Sugar	107	92	155	135	138	140		150¶	137	149
		Hemoglobin	90		92	84	92	92		89¶	90	93
10	23	Minute			19	29	38	49	62	74	109	154
		Sugar	92	97	120	137	143	160	170	170	123	118
		Hemoglobin	93	93	85	99	81	91	88	84	80	82
11	24	Minute			19	29	43	49	64	79	109	149
		Sugar	94	89	145	115	157	143	144	146	156	161
		Hemoglobin	103	104	85	85	90	93	84	84	84	87

TABLE 1—*Continued*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
EXPERIMENT	DOG	ITEM	INITIAL VALUE		15-25 (20 ± 1) MINUTES	25-35 (28 ± 3) MINUTES	35-45 (39 ± 2) MINUTES	45-55 (50 ± 1) MINUTES	55-65 (62 ± 2) MINUTES	65-85 (75 ± 3) MINUTES	85-125 (111 ± 6) MINUTES	125-165 (150 ± 5) MINUTES
			Jugular	Saphenous								
12	25	Minute				26	36	49	62	74	109	149
		Sugar	116	113		182	196	166	155	141	178	176
		Hemoglobin	99	96		93	89	89	93	102	94	110
13	13	Minute			19	29	39	52	59	74	114	150
		Sugar	82	78	121	149	154	150	162	164	165	680‖
		Hemoglobin	95		78	70	71	66	66	70	73	74
14	25	Minute			19	33	39	49	63	77	111	149
		Sugar	105	106	145	142	135	133	119	129	130	125
		Hemoglobin	100	100	68	95	98	85	94	94	80	78
15	15	Minute			19	29	39	49	63	76	116	149
		Sugar	133	142	191	163	166	175	178	163	172	177
		Hemoglobin	88	85	74	72	74	73	79	70	70	70
16	26	Minute			22	33	37	53		66	109	154
		Sugar	84	79	127	119	140	145		145	138	129
		Hemoglobin	"100"	105	100	103	110	95		92	98	92
17	21	Minute				32	39	49	62	74	109	144
		Sugar	133	113		165	155	163	184	173	156	133‖
		Hemoglobin	83	83		81	79	83	81	80	85	82
18	26	Minute			22	30	40	50	64	84	110	156
		Sugar	95		133	150	163	150	168	163	170	177‖
		Hemoglobin	85		82	91	82	85	78	90	89	95
19	31	Minute			19	29	39		59	74	124	
		Sugar	133	126	202	225	230		207	205	215	
		Hemoglobin	"100"	92	100	99	100		101	98	103	
20	32	Minute			19							
		Sugar	108	114	150							
		Hemoglobin	"100"	100	95							
21	33	Minute			20							
		Sugar	162	173	245							
		Hemoglobin	97	90	88							
22	34	Minute			19							
		Sugar	137	134	185							
		Hemoglobin	"100"	103	96							

TABLE 1—*Concluded*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	((13)
EXPERIMENT	DOG	ITEM	INITIAL VALUE		15–25 (20 ± 1) MINUTES	25–35 ((28 ± 3)) MINUTES	35–45 (39 ± 2) MINUTES	45–55 (50 ± 1) MINUTES	55–65 (62 ± 2) MINUTES	65–85 (75 ± 3) MINUTES	85–125 (111 ± 6) MINUTES	125–165 (150 ± 5) MINUTES
			Jugular	Saphenous								
23	34	Minute			21	33	43	53	58	75	109	150
		Sugar	100		179	168	206	208	206	199	226	237
		Hemoglobin	"100"		98	101	97	97	97	95	97	100
24	35	Minute			21	28	39	50		73	110	151
		Sugar	101		131	150	140	129		140	165	132
		Hemoglobin	"100"		92	93	86	92		94	88	104
25	36	Minute			20	31	39	52	63	75	109	148
		Sugar	101		158	165	153	142	143	168	159	164
		Hemoglobin	"100"		86	92	86	89	90	88	91	90

* Mean of 129 mgm. at 125 min. and 124 mgm. at 150 min.
† Mean of 131 mgm. at 90 min. and 146 mgm. at 120 min.
‡ Mean of 161 mgm. at 130 min. and 131 mgm. at 150 min.
§ Mean of 156 mgm. at 94 min. and 147 mgm. at 113 min. and 147 mgm. at 120 min.
¶ Mean of 140 mgm. at 72 min. and 160 mgm. at 81 min.; hemoglobins, 87 and 90.
‖ Not included in further calculations. See protocols.

TABLE 2

Average elevation of blood sugar above fasting level during continuous intravenous injection of glucose at the rate of 0.7 gram per kilo per hour. Dogs

MINUTES AFTER BEGINNING INJECTION	NO. OF EXPERIMENTS	NO. OF DOGS INCLUDED	MEAN ELEVATION ABOVE FASTING LEVEL. MGM./100 CC.
0 (Fasting)	25	13	0 (106±20 = fasting level)
20±1	15	12	50±16
28±3	22	11	48±18
39±2	22	11	55±23
50±1	19	10	52±28
62±2	16	10	53±28
75±3	19	11	60±38
111±6	21	11	56±38
150±5	17	10	58±49

average curve fails to show one characteristic of the individual curves, the definite irregularities that occur in the plateau period. Typically, following the initial rise in the first 20 to 30 minutes there is a prompt fall, which, however, does not continue, but is replaced by a horizontal course or another rise. Up to the point where this fall ceases these

curves are quite similar to the ordinary alimentary curves and are perhaps determined by the same factors. Beyond this point an added factor or a change in those already at work must be sought in one or the other type of curve.

The influence of emotion on the level of fasting blood sugar is well known. We have accumulated 7 experiments in which no emotion was displayed and no adequate cause for emotion occurred other than the venepuncture, a procedure to which dogs are usually insensitive. These are experiments 3, 5, 6, 7, 13, 23 and 24. Of these, all the curves are of the usual type except those of experiments 6 and 7. Experiment 7 furnishes the only curve of the entire series that falls as does an alimentary blood sugar curve. Experiment 6 is similarly distinguished by furnishing the only curve that rises rapidly during its entire course. With the unusually high curve (no. 6) omitted, the others show an average rise above fasting level of the same magnitude as those of the full series, with the same plateau formation. Emotion cannot be identified as the factor that causes the elevation to persist.

A rise or fall of blood sugar concentration can be due only to a change in the dilution of the blood, or to a difference in the relative rates of sugar intake into and disappearance from the blood. Dilution of the blood, as indicated by changes in hemoglobin per cent, occurs in these experiments, and has been shown to occur after alimentary administration of glucose, as recently by John (18), but in neither case does it materially affect the course of the curve.

Since in these experiments the rate of intake is constant, a general rise in the curve can occur only when the rate of disappearance is less than the rate of intake; a fall in the curve, on the other hand, occurs only when the rate of disappearance exceeds the rate of intake. The general course of the blood sugar curve during these experiments is explained by the statement that as the injection continues after its initiation the rate of disappearance rises, usually within the first half-hour, to a level equal to, and usually at first even above, the rate of intake, and approximately equals the rate of intake thereafter. How long the two rates would continue equal, and whether a slight excess in the rate of disappearance would ultimately bring the blood sugar down to its fasting level, remains to be determined. The experiments of Staub and of Boe with repeated doses of glucose by mouth over periods of several hours would lead us to look for an ultimate fall of blood sugar. On the other hand, the one experiment of Woodyatt referred to, revealed no fall in level during 5 hours of injection.

Up to and usually shortly beyond the apex, the alimentary blood sugar curve runs a course quite similar to those during intravenous injection. The apex of the alimentary blood sugar curve, interpreted in the light of our

results, simply marks the moment at which a rising rate of disappearance reaches and passes the rate of absorption from the gut. The theory of a threshold of sugar storage which is set at a level just below the renal threshold and which comes into play at the apex of the blood sugar curve, thus accounting for the fall thereafter (7), is clearly not supported, nor is the suggestion of a sudden discharge of insulin at the apex of the curve (12).

It is of interest to speculate, however, upon what rate of discharge of insulin might occur in these experiments. That the quantity liberated at any one time is for fairly prompt use is indicated by the experiment of Hédon (19) who was able to clamp off the blood supply of a pancreas transplant by pinching the vessels through the skin and observed a rise in fasting blood sugar in 5 hours to 183 mgm. per 100 cc., and by the experiment mentioned by Banting (20) in which a glucose tolerance test immediately after pancreatectomy gave a diabetic curve. Also that the insulin used and the sugar disposed of bear a quantitative though not a simple relationship, in normal subjects at any rate, is likely. An increase in the number of grams of sugar disappearing from the blood per unit of time, so far as it is due to storage or burning of sugar, should be accompanied by an increase in the quantity of insulin used per unit of time. If during intravenous injection the rate of disappearance of sugar gradually rises to a maximum shortly after the apex of the blood sugar curve is passed, we should expect the rate of utilization and probably the rate of liberation of insulin to follow a similar course. A curve of the rate of disappearance may therefore prove to represent the actual rate of insulin discharge. The extra quantity necessary to convert a blood sugar curve of the plateau type into a curve that would fall to the fasting level by the end of two hours and a half, if proportional to the extra quantity of sugar that must disappear to bring the curve down, would be only about 3 per cent more than necessary to explain the course of the plateau curves as they stand.

We have assumed that in the alimentary curve the first part of the fall past the apex is due to the same excessive rate of disappearance that accounts for the short fall usually seen in the curves during intravenous injection. In the intravenous experiments after the blood sugar fall has progressed a variable distance the disappearance rate also falls, reaches the level of the intake rate and halts the further fall of the blood sugar. The alimentary experiments may be an exception to this in that their disappearance rate remains above the rate of intake without falling or with little fall; or the fall of disappearance rate may perhaps occur but in the presence of a rate of intake which is also declining, and rapidly enough to prevent its being overtaken by the falling rate of disappearance. There are considerations that favor both possibilities, and, indeed, a

combination of the two may eventually prove to be the actual cause of the falling portion of the blood sugar curve. Possible causes for a maintained high rate of disappearance are: *a*, a higher concentration of sugar in the portal blood during absorption as compared with intravenous injection at the same rate; *b*, the possibility of action by the intestinal mucosa upon the sugar, converting it into a more reactive form (21); *c*, the possibility that in some other way sugar taken by mouth is partly converted into a different form, whereas sugar injected intravenously is not (22); *d*, the possibility that intestinal absorption may set free some hormone (e.g., secretin) or initiate some nerve impulse that stimulates more active sugar storage.

On the other hand, evidences favoring a declining rate of intake into the blood as a factor in the fall of the alimentary blood sugar curve are: *a*, the finding of Staub, Boe and Maclean and De Wesselow that the fall may be delayed by giving repeated doses of glucose; *b*, the finding of Staub, Hansen and Maclean and De Wesselow that the fall may be delayed by giving larger doses of glucose; *c*, the finding of Boe that a more dilute solution of glucose by mouth resulted in a lower curve than the same dose in concentrated solution, in connection with the findings of Beeler, Bryan, Cathcart and Fitz (15) that glucose in hypertonic solution by mouth becomes diluted before it has all left the stomach.

SUMMARY

1. Work is reviewed which indicates that: *a*, the level of hyperglycemia varies with the rate of intake of sugar into the blood; *b*, the duration of hyperglycemia varies with the quantity of glucose administered, and, therefore, presumably with the duration of the intake of sugar into the blood; and *c*, some unidentified, possibly third factor, may restore the blood sugar to its fasting level, in a variable time, during repeated administrations of glucose by mouth.

2. Experiments in which a constant rate of intake of sugar into the blood was maintained for 2½ hours by intravenous injection at the rate of 0.7 gram of glucose per kilo per hour in dogs, yielded blood sugar curves that remained elevated during the injection period.

3. During such continuous injection hemoglobin percentage changes indicate that dilution of the blood occurs, most marked in the first portion of the experiment, but not sufficient essentially to affect the course of the curve.

4. Under the conditions of these experiments the forms of the blood sugar curves must be ascribed essentially to changes in rate of disappearance of sugar from the blood.

5. The factors of rate of disappearance and rate of intake are discussed as determining the course of the alimentary blood sugar curve.

Acknowledgments are made to Prof. R. G. Hoskins, in whose laboratory this work was done, for his unfailing encouragement and helpful criticism, and to several students who from time to time have rendered technical assistance.

BIBLIOGRAPHY

(1) Woodyatt: Journ. Biol. Chem., 1920, xli, xliii (Proc.).
(2) Macleod and Fulk: This Journal, 1916, xlii, 193.
(3) Allen and Wishart: Journ. Biol. Chem., 1920, xlii, 415.
(4) Woodyatt: The Harvey Lectures, 1915–16, xi, 326.
(5) Eadie: This Journal, 1923, lxiii, 513.
(6) Staub: Zeitschr. f. klin. Med., 1921, xci, 44.
(7) Maclean and Dewesselow: Quart. Journ. Med., 1921, xiv, 103.
(8) Hansen: Acta Med. Scand., 1923, Suppl. iv.
(9) Boe: Biochem. Zeitschr., 1914, lviii, 106.
(10) Bang: Die Blutzucker, cited by Boe (9).
(11) Traugott: Klin. Wochenschr., 1922, i, 892; Zeitschr. f. d. gesammt. exper. Med., 1923, xxxi, 282.
(12) Foster: Journ. Biol. Chem., 1923, lv, 303.
(13) Meyer-Bornicke: Zeitschr. f. d. gesammt. exper. Med., 1923, xxxi, 341.
(14) Killian: Proc. Soc. Exper. Biol. and Med., 1920, xvii, 91.
(15) Beeler, Bryan, Cathcart and Fitz: Journ. Metab. Res., 1922, i, 549.
(16) Myers and Bailey: Journ. Biol. Chem., 1916, xxiv, 147.
(17) Palmer: Journ. Biol. Chem., 1918, xxxiii, 119.
(18) John: Journ. Metab. Res., 1922, i, 498.
(19) Hedon: Arch. internat. d. Physiol., 1923, xxi, 8.
(20) Banting: Journ. Mich. State Med. Soc., 1923, xxii, 113.
(21) Hewitt and Pryde: Biochem. Journ., 1920, xiv, 395.
(22) Hewitt and Desouza: Biochem. Journ., 1921, xv, 667.
(23) Winter and Smith: Journ. of Physiol., 1922, lvii, 100.

Protocols. *Experiment 1*: 6/12/23. Dog 13, F, mongrel bull, 19.7 kilos. Fasting 20 hours. Much difficulty in entering jugular vein, dog showing signs of pain. At 40–60 minutes, shivering. Total glucose solution injected, 72 cc.; saline solution injected, 20 cc. Time, 1 hour.

Experiment 2: 6/15/23. Dog 15, M, mongrel collie, 13.2 kilos. Fasting 21–22 hours. A bit restless for first few minutes and at one time toward the end; otherwise quiet. Some evidence of pain on entering jugular. Total glucose injected, 115 cc.; saline, 87 cc.

Experiment 3: 6/15/23. Dog 21, M, moderately old, long haired black, 13.75 kilos. Fasting 16 hours. At 50–60 minutes, no injection, 60 to 75 minutes, injection at increased rate to catch up with schedule. Dog quiet throughout experiment except for occasional moving of feet. Total glucose injected, 120 cc.; saline, 60 to 80 cc.

Experiment 4: 6/19/23. Dog 13 (see exper. 1). Fasting 16 hours. Jumped on inserting needle. No signs of emotion thereafter. At 75 minutes, considerable difficulty in reëntering vein, with signs of pain. At 90 to 100 minutes, restless, respirations increased. At 115 minutes, reintroduction of needle. Total glucose injected, 173 cc.; saline, 35 to 50 cc.

Experiment 5: 6/20/23. Dog 21 (see exper. 3). Fasting 15 hours. Wagged tail at intervals throughout experiment. At 105 to 113 min., no injection; 113 to 120 minutes, injection more rapid to catch up with schedule. Total glucose injected, 120 cc.; saline, 70 cc.

Experiment 6: 6/26/23. Dog 21 (see expers. 3, 5) 13.5 kilos. Fasting 20 hours. Seemed to be annoyed by flies occasionally, but otherwise quiet, and wagged tail throughout experiment. Total glucose injected, 118 cc.; saline, 44 cc.

Experiment 7: 6/27/23. Dog 15 (see exper. 2) 14.5 kilos. Fasting 14 to 15 hours. Unsatisfactory control of injection rate permitted quantity injected to be 3 cc. ahead of schedule at 46 minutes, on time at 53 minutes, 5 cc. ahead at 69 minutes, on time at 78 minutes, 2 cc. behind at 82 minutes, on time at 113 minutes, and 2 cc. ahead at 157 minutes, the end of the experiment. Dog absolutely quiet throughout. Total glucose injected, 133 cc.; saline, 60 cc.

Experiment 8: 6/29/23. Dog 23, F, tan, short hair, cur, 17.2 kilos. Fasting 14 hours. Occasional gentle whining during the first hour, dozing thereafter. Total glucose injected, 156 cc.; saline, 35 cc.

Experiment 9: 7/2/23. Dog 24, M, Black and white, long hair, 16.3 kilos. Fasting 20 hours. Was attacked by another dog just before the experiment. Injection rate 0.74 gram per kilo per hour, due to error in weighing dog. Quiet throughout experiment. Total glucose injected, 150 cc.; saline, 28 cc.

Experiment 10: 7/3/23. Dog 23 (see exper. 8) 17.0 kilos. Fasting 20 to 21 hours. Occasional gentle whining during first hour, quiet thereafter. At 11 to 117 minutes, no injection; 117 to 119 minutes, caught up with schedule. Total glucose injected, 151 cc.; saline, 40 to 50 cc.

Experiment 11: 7/5/23. Dog 24 (see exper. 9) 16.3 kilos. Fasting 20 hours. Appeared somewhat nervous for first half hour, quiet thereafter. Total glucose injected, 143 cc.; saline, 35 cc.

Experiment 12: 7/6/23. Dog 25, F, tan, short hair, cur, 17.6 kilos. Fasting 20 hours. Difficulty in entering both veins. Quiet throughout experiment except for occasional momentary restlessness. At 68 minutes, lost 2 cc. of injection by disconnecting tubing; this not caught up. Total glucose injected, 154 cc.; saline, 37 cc.

Experiment 13: 7/9/23. Dog 13 (see expers. 1, 4) 19.2 kilos. Fasting 48 to 50 hours. Quiet throughout experiment except for occasional shivering, and vomited once during latter half of period. Total glucose injected, 168 cc.; saline, 16 cc.

The blood taken at 150 minutes contained 680 mgm. of reducing substance per 100 cc. This finding was checked by a third determination on more of the same blood. Calculation of the probable total blood volume, total blood sugar on the basis of the concentration of this sample, and of the quantity of glucose injected since the last blood sample was taken, showed that so high a level could have been reached only if there had been a flow of sugar into the blood from the tissues as well as from the injection. This value, 680 mgm. per 100 cc., is therefore not used in calculating the average of all curves.

Experiment 14: 7/10/23. Dog 25 (see exper. 12) 16.5 kilos. Fasting 12 to 22 hours. At 65 to 75 minutes, shivering; 90 minutes, nausea. At 105 minutes, lost 4 cc. by disconnecting tubing. Caught up 2 cc. at 105 to 111 minutes, remaining 2 cc. behind schedule thereafter. At 140 minutes, a trifle restless otherwise quiet throughout experiment. Total glucose injected, 141 cc.; saline, 57 cc.

Experiment 15: 7/11/23. Dog 15 (see expers. 2, 7) 14.7 kilos. Fasting 14 to 20 hours. Restless at times, throughout experiment. Total glucose injected, 128 cc.; saline, 24 cc.

Experiment 16: 7/11/23. Dog 26, F, airedale, 13.4 kilos. Fasting 18 to 24 hours. Trained only one day before experiment. Quite restless, whining, and trying to get up at intervals for first 40 minutes, quiet thereafter. At 146 minutes, lost 2 cc. of injection, not caught up. Total glucose injected, 116 cc.; saline, 32 cc.

Experiment 17: 7/12/23. Dog 21 (see expers. 3, 5, 6) 14.6 kilos. Fasting 14 hours. At 23 to 25 minutes, and at 28 to 30 minutes, no injection, with loss of 4 cc. by delay and changing needle. Remained behind schedule up to 90 minutes, when caught up 2 cc., and 2 cc. more at 97 minutes. Dog whining at intervals throughout second hour. At 134 minutes, injection ceased. Last sample at 145 minutes, not used in average of all curves owing to 11 minute delay. Total glucose injected, 115 cc.; saline, 35 to 40 cc.

Experiment 18: 7/24/23. Dog 26 (see exper. 16) 14.2 kilos. Fasting 15 hours. At 90 to 110 minutes, very restless, trying to get up. Total glucose injected, 125 cc.; saline 77 cc. Last blood taken 6 minutes after injection ceased, and not used in average of all curves because of this delay. The high value of this sample in spite of this delay may be related to the period of marked restlessness just preceding.

Experiment 19: 8/25/23. Dog 31, M, tan, mongrel collie, 19.3 kilos. Fasting 20 hours. Short training, 2 days. Made violent effort to get up when jugular needle inserted, quiet thereafter until 50 to 60 minutes, when difficulty in reinserting needle, with signs of pain. At 110 to 125 minutes, difficulty in reinserting needle, considerable restlessness, quiet thereafter; 20.8 per cent solution of glucose injected. Total glucose injected, 140 cc.; saline, 17 cc. Time, 124 minutes.

Experiment 20: 9/24/23. Dog 32, M, white, short hair, 10.5 kilos. Fasting 36 hours. Quiet. Total glucose injected, 12 cc.; saline, 34 cc. Time, 20 minutes.

Experiment 21: 9/24/23. Dog 33, F, black, long hair, young, 9.55 kilos. Fasting 24 hours. Quiet. Total glucose injected, 11 cc.; saline, 40 cc. Time, 20 minutes.

Experiment 22: 9/28/23. Dog 34, M, large tan mongrel collie, 22.6 kilos. Fasting 15 to 16 hours. Trained only one day, and rather restless during experiment. Total glucose injected, 32 cc.; saline not recorded but less than 50 cc. Experiment lasted 25 minutes, during which 14 samples were taken. Only the fasting and the 19 minute samples are recorded in table 1.

Experiment 23: 10/24/23. Dog 34 (see exper. 22), 21.1 kilos. Fasting 16 to 20 hours. Quiet throughout experiment. Number of samples taken, 36, of which only those taken at times corresponding to the usual schedule of samples, are recorded in table 1. Total glucose injected, 160 cc.; saline, 175 cc. The glucose solution was 23 per cent instead of the usual 20 per cent.

Experiment 24: 11/4/23. Dog 35, F, white, long hair, 18.65 kilos. Fasting 16 to 20 hours. Ate a piece of dog biscuit half an hour before the experiment. Presumably no effect on blood sugar in that time, as the initial sample contained 100 mgm. of reducing substance per 100 cc. Quiet throughout experiment. Number of samples taken, 40, of 2 cc. each, during 159 minutes. Only those taken at times corresponding to the usual schedule of samples, are recorded in table 1. Analyses run on 1 cc. portions of blood. Total glucose injected 150 cc.; saline 210 cc. The glucose solution was 23 per cent instead of the usual 20 per cent.

Experiment 25: 11/9/23. Dog 36, M, tan, long hair, 13.7 kilos. Fasting 16 to 20 hours. Vigorous resistance on inserting needle, quiet thereafter. Number of samples taken, 39, of 2 cc. each, in period of 155 minutes. Only those taken at times corresponding to the usual schedule of samples are recorded in table 1. Analyses run on 1 cc. portions. Total glucose injected, 108 cc.; saline, 217 cc. The glucose solution was 23 per cent instead of the usual 20 per cent.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XIX. A Hitherto Unknown Function of the Thymus

OSCAR RIDDLE

From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Received for publication February 27, 1924

Recorded details of the reproductive history of large numbers of doves and pigeons bred during several years have made it clear that among these animals a notable though variable percentage of individuals show, either throughout their life or during a part of it, some pronounced abnormality of reproductive functioning. Such details of the reproductive history as have been continuously recorded in our studies are impossible of acquirement in mammals and the records obtained by us are perhaps more complete and extensive than have yet been obtained for any vertebrate. Even a cursory inspection of these data leads to the conclusion that *these abnormalities belong to several obviously different types.* During the last four years we have made opportunity to study these reproductive disorders, since most of them had appeared in connection with our prolonged studies on sexuality in the ova of pigeons, and had there introduced vexatious irregularities for which there was no known explanation.

The nature of these several disorders, not including those obviously associated with hybridity, at first suggested that they might have their origin in one or all of the three following sources: *a*, infection; *b*, defective nutrition; *c*, dysfunction of one or another organ of internal secretion. An incomplete though serious inquiry into the relationship of "infection" to these abnormalities soon led us to discard this as a probable cause of the more frequent types of disorder. In collaboration with Mr. Embree R. Rose (unpublished data) the second suggestion was adequately tested by the addition of measured amounts of vitamines A, B and C, of a long list of inorganic salts, and of various amino acids and proteins, to the normal diet of the affected birds. These tests were conducted during nearly two years on birds showing the various types of reproductive disorder and the results made it clear that the principal disorders studied by us are not traceable to a diet deficient for adult reproducing birds.

On the other hand, the early stages of our inquiry into the possible endocrine basis of the abnormalities—the last of the three suspected causes to be studied—clearly indicated that at least some of the types of

disorder in question are in some way associated with a dysfunction of one or another of the incretory organs. In general, however, few things are as yet clear and definite beyond the point just stated. Doubtless this is necessarily so, because of the numerous, complex and still doubtful interrelationships of the ductless glands—and the even less understood relationship of most of these glands to the processes of reproduction in any animal. The several results of our studies on this subject, however, clearly suggest that many or most of the incretory organs bear a peculiarly intimate relation to reproduction. Also, that some of these relationships may be more easily and perhaps more successfully studied in an animal which retains the primitive vertebrate mode of reproduction than is possible in the mammal where the reproductive system has undergone extensive and anomalous transformation.

The five preceding papers of this series have considered the relation of suprarenals, and the blood sugar, to one or another aspect of normal reproduction. As a first report upon the present group of investigations on the cause or basis of reproductive abnormalities we wish to present results which apparently show that thymus tissue, or one or another of its derivatives, is essential to the secretion of the egg-envelopes—egg albumen and egg shell—by the vertebrate oviduct. Further, that results obtained from thymectomy in reproducing birds, though limited in application and in quantity at present, supply only confirmation that this is a rôle of the normal thymus. If these results may be considered a complete demonstration the new data on this particular point disclose a hitherto unknown function of the thymus. They also seem to supply for the first time a demonstration of the truly endocrine nature of this organ.

On material and on the general effects of thymus dosage. A restricted group, consisting of five female individuals, has been found in which the eggs produced were of abnormally small size though provided in probably all cases (except no. 4) with yolks of normal size. The abnormal egg size thus results from the secretion of abnormally small amounts of albumen and of shell. The albumen deficiency, usually quite evident, is easily determined after obtaining weights of the whole egg and of the enclosed yolk. The shell deficiency is often entirely obvious (soft shells); in less obvious cases it is readily measured by the increased rate at which the shell permits the loss of water vapor from the egg. The thinner the shell the higher this rate of loss. Dove embyros developing in eggs with a high rate of loss are rarely viable (1). In the most extreme cases double and movable air-chambers have often been present—thus indicating some failure or inadequacy of the shell membranes. Also, in the most extreme case (no. 1) qualitative changes have been observed in the (diminished) egg albumen. This "albumen" was not coagulated by heat, and was probably largely ovomucoid.

This type of abnormality is perhaps further characterized by some points less clearly connected with egg-envelopes. The number of eggs in the clutch or pair is frequently reduced from two to one. It is conceivable that the imperfectly functioning oviduct—as shown by its deficient secretions—is less efficient in catching all of the ovulated ova, and permits some of them to pass into the body cavity. This would result in "single" eggs, as would also a very different situation, namely the growth and ovulation of single instead of paired ova by the ovary. In none of the numerous birds tested by thymus feeding can we find any definite effect upon the rate or promptness of ovulation cycles. Four of the five birds of table 1 (case 4 excepted) showed one or another degree of obvious defect in the keel of the sternum. This bone is the most easily observed skeletal structure of the bird. Curvatures or depressions in its ventral aspect are not infrequent among doves and this observation may or may not be significant in connection with this group of birds. Since deficient or abnormal calcification seems to be involved this observation may be of some interest here. It remains to note that three of the five individuals which developed this group of reproductive abnormalities (sternum not considered) had earlier shown nearly or quite complete normality in these several respects; and that when killed for the necessary examination the birds thus affected have shown, without exception, abnormally small thymi. The three cases (nos. 1, 2, 3) just mentioned were blond ring doves. Cases 4 and 5 were also blond ring doves chiefly, but of hybrid composition, and this fact probably partly or wholly accounts for their lack of complete reproductive normality at the beginning of egg production.

As far as this was practicable the attempt was made to administer in an effective form the several endocrine products, including the thymus, to one or more birds representative of each of the several types of reproductive disorder; also to study the effects of each such product on a few wholly normal animals. For several of these glandular products the study included not only a test of fertility or hatching power but also a test of possible influence on the size of the ova (yolks) produced; the study with any single product in any single bird usually covered a period varying from a few months to two years. In most cases a "period" of dosage was continued until about 6 eggs had been laid under dosage, and this period was followed by a control period during which another 6 eggs were produced (under blank dosage daily with empty gelatin capsules).

On the above plan desiccated thymus (Parke, Davis & Co.) in gelatin capsules was administered to 29 females. Six of these were normal birds given smaller or larger dosage during nearly one year and in which egg size and yolk size were continuously measured. In these normal birds, and in others of various abnormal types, yolk size was either wholly unaffected by thymus dosage or it was very slightly increased. Five of the 23 remaining birds were of the type discussed above. Of

the other 18 birds showing reproductive disorder, but whose disorders were unaffected by thymus treatment, two had also other organic disease and produced only one or two eggs after beginning thymus dosage.

The other 16 treated and adequately tested birds belonged chiefly to groups whose reproductive disorders were characterized as follows: *a*, Single clutches, asymmetrical eggs, dead embryos, good shells, eggs and yolks of normal size. Dosage here had no effect. *b*, Single clutches, small eggs and yolks, normal shells. No effect. *c*, Hybridity, singles, prematures, small eggs and yolks. No effect. *d*, Retarded ovulation rate (autumn). No detectable effect. *e*, Numerous singles from "masculine" females, thin shells, prematures, yolks small, eggs normal or sub-normal for yolk size. Probably no effect (disorders increased in the two cases studied). *f*, Eggs of clutch or pair with reversed or abnormal size relations, single clutches, laying from perch, prematures, thin shells. Probably no effect. *g*, Eggs of clutch with reversed size relations, rare singles, egg size somewhat reduced (yolks?), extra thick shells. No effect.

Three of the five cases which responded to thymus administration were tested prior to the thymus dosage with a number of nutritional or endocrine substances. The results were negative in all cases. The other two cases were tested after thymus dosage with other endocrine products. We list below, in the order of their administration to each bird, the several substances used. All of the substances listed were administered once or twice daily in an appropriate manner and dosage, and in such quantity during a sufficiently prolonged period as to give a satisfactory test.

Case 1. Vitamine B (yeast), vitamine C (tomato), K_2HPO_4, whole milk powder (klim), *thymus*. Case 2. Water solutes from fresh portland cement, HCl, vitaphos, vitamine C (tomato), vitamine A (spinach), As_2O_3, $Na_2Si_4O_9$, calcium lactate, K_2HPO_4, parathyroid, *thymus*. Case 3. Vitamine A (codliver oil), vitamine C (orange), cystine, K_2HPO_4, whole pituitary, parathyroid, *thymus*. Case 4. *Thymus*, whole suprarenal. Case 5. *Thymus*, suprarenal cortex, insulin.

In the case of products administered soon after previous thymus dosage it is less easy to determine the effect produced since, as noted elsewhere, the improvement effected by the thymus tends to persist long after its dosage is discontinued. The data obtained in these few last-named tests fail to indicate any effect, but they also had less opportunity to produce effects than had the thymus dosage. The entire group of tests would seem to make it practically certain that there exists in thymus tissue some component not present in any of the substances used prior to thymus dosage at least and that this thymus component exerts a specific action upon the deficient reproductive secretions.

The most important observation in this report—after finding a group of birds with the syndrome just described and showing that the syndrome is not the result of dietary deficiency nor of infection —is that the oral administration of daily doses of only 5 to 20 mgm. of desiccated thymus gland has removed, partially or completely, their chief reproductive deficiencies in all tests made upon this special type of abnormality. The very prompt return of the secretion of albumen and shell to or toward the normal has been particularly notable in all cases. Other desiccated tissues

have wholly failed in the few tests made with them. Further tests of this nature will of course be made when new cases of this rare type of defect shall again be found.

TABLE 1

Summary of effects of thymus dosage on the secretion of egg-envelopes and on other abnormalities

CASE NUMBER; AND BIAD	PERIOD; DOSAGE OR CONTROL	DOSAGE	NUMBER OF EGGS	AVERAGE WEIGHT		ALBUMEN / YOLK	RATE OF LOSS PER HOUR	PER CENT "SINGLE CLUTCHES"	PER CENT HATCHS	THYMUS WEIGHT
				EGGS	YOLKS					
		mgm.		*grams*	*grams*		*mgm.*			*mgm.*
(1)	Control		10	4.67	(2.125)	1.35	13.4+	82	0*	
A622	Dosage	20	*9*	*8.87*			*3.1*	*0*	*78*	
	Control		22	8.49	(2.010)	3.47	4.7	0	60*	
	Control		22	7.72	(2.247)	1.99	4.2	43	45*	
	Control		6	6.68	(2.287)	1.87	3.2	80	33*	
	Dosage	20	*5*	*9.05*	*(2.287)*	*3.05*	*3.7*	*33*	*100*	
	Control		2	9.52	2.351	3.05		0		31.5
(2)	Control		9	6.79			50.2+	20	0†	
B629	Dosage	5	*5*	*8.28*			*5.9*	*75*	*60*	
	Control		7	8.47			7.0	25	71	37.6
(3)	Control		7	7.33			4.0	25	43	
152	Dosage	10	*8*	*7.63*			*3.5*	*0*	*62*	
	Control		8	7.54			4.0	0	62	22.6+
(4)	Control		7	6.39			(2.7)+	25	29	
P843	Dosage	20	*4*	*7.11*			*2.7*	*0*	*50*	
	Control		7	7.18			3.1	25	?	25.5
(5)	Control		4	6.61				0	0	
T106	Dosage	5	*6*	*8.19*			*5.2*	*40*	*50*	
	Control		5	8.25			7.4+	0	40	
	Dosage	20	*7*	*8.75*			*4.5*	*0*	*86*	
	Control		6	8.95			4.4	0	83	(40.0)

* Air-chambers double and movable in some eggs.

† Three eggs with soft shell.

PRESENTATION OF DATA. *The data from thymus feeding in summary form.* The chief facts concerning the several effects of the administration of small amounts of thymus to five birds showing a fairly definite type of reproductive abnormality are shown in table 1. Several facts can be textually stated here but the reader is requested to examine all details of the tabulated data in the light of the analysis given below.

It will be observed that the average gross weight of the eggs laid during the control period which preceded the period of thymus dosage is smaller than that obtained under dosage in all of the seven comparisons provided in the table. It is also true that the gross egg weight obtained in the second control period (following dosage) is larger than the first control in all cases. The effects of the administration of thymus on total egg size therefore tend to persist beyond the actual period of its administration. Indeed, in some cases the first 6 eggs following dosage were heavier than the first 6 eggs during dosage. The effect of the thymus was greatest in those cases (1 and 5) in which the weight of the egg—or more accurately the amount of albumen—had been most reduced (concerning case 4, see below). With or without a consideration of yolk size (see below) it is evident therefore that in all cases the amount of albumen was increased *during* and *following* thymus dosage.

Under the "rate of loss" column it is shown that the highest rate, and therefore the thinnest shells, was found in the control preceding the first dosage in all of the four cases for which the data for rate of loss in this earliest control are available. The plus (+) signs attached to some figures of this column indicate that one or more eggs were soft-shelled, or so thin-shelled that they became broken before the rate of loss could be measured and if these could have been included the rate of loss would have been still higher than the rate indicated. In all except two cases (no. 2, and first dosage under 5) the rate of loss—i.e., the shell thickness—was brought within normal limits (2.5 to 5.0 mgm. loss per hour) during the period of dosage. The two exceptions are precisely those cases in which the smallest dosage (5 mgm.) was used, and even in these cases the values found closely approach the normal. This decreased rate of loss tended also to persist after discontinuance of dosage. It is therefore clear that the amount of shell substance was definitely and in most cases very greatly increased *during* and *following* the exhibition of thymus.

In the next column are given data for the "percentage of single clutches" produced at various periods with reference to dosage. The percentages given are for cases in which only one egg was laid instead of the usual two. One of the birds (no. 5) threw no single clutches during the period covered by the tabulation but our records show that it had previously done so. In four of the five periods of dosage of the other four birds the percentage of "single clutches" was decreased during thymus dosage. The exception is found in the bird given the smallest dosage (5 mgm.). The administration of thymus therefore tends to correct this abnormality when it occurs as a part of this syndrome.

The "percentage of eggs hatched" is shown in the table to have been increased during the dosage period in all of the seven tests. In the control period following dosage an increase over the earlier control is main-

tained in all of the five tests made. The percentage hatched was usually higher during dosage than in the control period following dosage. In calculating the "percentage hatched" a deduction was made for eggs taken at once for the determination of yolk size. Also this percentage is sometimes based on a higher number of eggs than those numbered and weighed, since some were soft-shelled, broken before weighing, etc. An uneven number of eggs in the fourth column does not necessarily indicate "single clutch" eggs for similar reasons. It is clear that the feeding of either larger or smaller amounts of thymus increased the hatching capacity of eggs produced by birds of this particular type.

It is now necessary to consider the question of the size of yolks in relation to the egg-envelopes. These two things, of course, form the total weight of the egg; and where yolk weight is given (tables 1 to 4) this weight subtracted from the egg weight gives the quantity of egg-envelopes secreted. The total weight of a complete normal shell of the ring dove egg has earlier been shown to be usually less than 0.5 gram (2). The amount of egg albumen (and total egg-envelopes) secreted is normally dependent first of all upon the size of the yolk which is enclosed. For each gram of yolk about 3.0 to 4.0 grams of albumen are secreted but the smaller the yolk the higher is the ratio of albumen to yolk (2). This column of table 1 shows that in case 1 only 1.35 grams of envelope was being secreted prior to the first thymus dosage, and only 1.87 grams prior to the second dosage period. Following thymus dosage, however, these ratios were increased to 3.47 and 3.05 grams respectively. Some yolk weights are enclosed in parentheses because they represent fewer eggs than do the egg weights which precede them in their row in the table. Also, the figures of the "albumen ÷ yolk" column are derived from the weights of those particular eggs whose yolks are included in the comparison, and not from the weights of all eggs belonging to that dosage or control period. Some yolk weights were obtained from eggs which had tested infertile and a correction has been made for a change in weight during the two or three days necessary for that test.

The size of the yolks produced by other birds than case 1 is known, at least approximately, from yolks weighed either before or after the periods included in the table, or from the inspection of the size of the yolks as found in the infertile, soft-shelled, or broken eggs. In only one of these five cases were the yolks as well as the eggs abnormally small. In case 4, three yolks weighed nearest to (one was actually within) the period tabulated average only 1.417 gram. In this case therefore the normal or expected total egg size could be but little above 7.0 grams. Hybridity, as noted above, or still another complication in addition to the syndrome under consideration here, was probably involved in the production of unusually small yolks by this bird. But that the egg-envelopes were disproportionately small is indicated by the several results of the thymus dosage.

The last column of table 1 gives the thymus weights found for each of the six birds when killed for examination. Since certain diseases and inanition were known rapidly to reduce the size of the thymus in pigeons the attempt was made to kill these birds while in their normal state of health as shown by the continued production of eggs. Nevertheless, the condition found at necropsy in case 3, a probably tuberculous infection of the liver and a wing-joint, had possibly effected some reduction of the thymus in this bird. This possibility is indicated by placing a plus (+) sign after the weight actually obtained. It will be seen that the weights of the thymi of these five birds ranged from 23 to 40 mgm. A comparison of these figures with the normal or "expected" size for these birds is a complicated matter which will next be considered.

Control data on thymus size. Our own unpublished data for thymus weight in approximately one thousand doves and various pigeon hybrids are available for a determination of the normal size, and for a knowledge of the factors which influence size, of the pigeon thymus. Jolly and Levin (3), (4) pointed out that thymic involution in birds does not occur until a far later period than sexual maturity. We have learned that although the thymus by no means disappears in the adult pigeon, it does become markedly and progressively reduced in old age. Age is therefore a factor of importance, and the exact age of every bird examined in any way by us is known. McCarrison (5) has shown that inanition, avitaminosis and septicemia speedily result in a reduction of thymus size in common pigeons, and we can confirm this effect of inanition and disease in both common pigeons and ring doves. The presence of advanced tuberculosis effects a particularly notable involution of the thymus. Sex probably introduces a difference, as was also noted by McCarrison, but the present data concern females only. Species having markedly different body size show corresponding differences in thymus size. Individuals of the same sex and species are of approximately similar size and any such size difference is here left out of account.

We have therefore evaluated the "expected" size of the thymi of the five birds involved in this study in the following manner: The ten birds most closely comparable with the three blond ring doves (cases 1 to 3) in sex, species, age and condition of health or disease at the time of killing were selected from the total of our available data at the time this was written. The average thymus weight obtained from these ten birds is used as a control for these three birds. The ten "control"—the best control I am able to obtain—had an average thymus weight of 76.3 mgm. The thymi of the three birds suspected of thymus deficiency, because of their having yielded to thymus treatment, average 30.6 mgm. Similarly, 10 birds were selected to conform to the two ring dove hybrids (cases 4 and 5). These younger birds showed an average thymus weight of 129.8 mgm. The

two affected and successfully treated hybrids give an average of 33.8 mgm. Only two of the above 20 control birds failed to show larger thymi than the largest of the treated group of five. In view of the wide temporary fluctuations to which thymus weight is subject this result clearly indicates abnormally small thymi in all of the birds which responded to thymus dosage.

It is possible to take as a second group of controls those birds which suffered from one or another type of reproductive disorder and were, like the five birds of interest here, dosed with thymus. Data for thymus size are available for 13 of these 16 birds and the average found for them is 91.4 mgm. Only two of this group failed to show larger thymi than the largest found in the group of five. It is true, however, that the group of 13 averaged considerably younger (36 mo.) than the group of five (61 mo.). On the other hand it is possible that some of the oldest of the latter group partly owed the functional and size deficiency of their thymi to the thymic involution which accompanies great age. It is nevertheless true that birds which at the time of necropsy were fairly young (25 mo.) as well as one that was quite old (99 mo.) are included in the group of five. It is further notable that these birds developed their reproductive disorders a. few months, or even two or three years, before reaching the age (at necropsy) assigned to them above.

Much might be said concerning the element of uncertainty involved in these "control" weights. In several females dead of disease we have found thymi even smaller than those found in these five successfully treated birds; but the known facts, and an obviously fair method of comparison, exclude these cases precisely because of their death from disease. The normal growth curve of the thymus in doves, and the effects of a variety of conditions upon its size, must be reserved for treatment in a subsequent paper. We think, however, that the data already given adequately show that the thymi of all the five successfully treated birds were abnormally small; and probably this abnormal size obtained during the whole period of their reproductive disorder.

It further seems wholly probable that the relative weights of the thymi in "control" and "affected" (successfully treated) birds do not adequately indicate the full extent of the reduction of actual thymic tissue in the five treated individuals; for, the latter had notably smaller glands and it almost necessarily follows that these smallest glands contained relatively more of the connective tissue inseparable from the gland at weighing than did the larger, less involuted glands. As prepared for weighing each of the two dove thymi, even when most reduced, usually retains its delicate enveloping connective tissue in a strand or thread approximatety 5 cm. long. Whatever may be the unmeasured sources of inaccuracy it is clear that *an abnormally small amount of thymic tissue was found* in each of the five birds which was favorably affected by thymus administration.

TABLE 2

Reproduction record of ♀ A622 during first four years—1917–1920

DATE	WEIGHT OF: Egg	Yolk or fertility	DATE	WEIGHT OF: Egg	Yolk or fertility	DATE	WEIGHT OF: Egg	Yolk or fertility
1917 (Early)			1918 (Early)			1919 (Late)*		
3/12	8.02	Hatched	1/24	8.79	(Lost)	12/1	7.82	2.165
3/14	8.52		1/26	8.56	2.146	12/3	7.66	2.195
4/6	9.00	2.213	2/20	8.96	2.200	12/10	7.61	2.073
4/8	9.16	2.255	2/22	9.28	2.347	12/18	7.13	1.956
5/17	8.90	2.068	3/25	8.23	2.148	12/20	7.81	2.175
5/19	9.35	2.308	3/27	8.32	2.145	12/26	7.43	1.971
Ave.	8.87	2.162(20)	Ave.	8.61+	2.100+ (14)	Ave.	8.07	2.140(14)
(20 eggs) No singles			(30 eggs) No singles			(39 eggs) 3 singles		
Complete record for 1920								
1/4	7.78	2.195	4/22	5.80	Infertile	9/4	4.78	2.068†
1/6	8.25	2.383	4/24	6.96	4-day embryo	9/10	4.83	1.995
1/12	6.83	1.843	4/30	5.99	7-day embryo	9/12	6.12	2.542
1/14	7.82	2.232	5/7	5.67	2-day devel.	9/22	5.05	2.565
1/20	7.23	2.046	5/15	6.33	7-day embryo	9/30	4.94	
1/22	7.60	2.190	5/17	6.96	1-day devel.	10/9	4.95	2.308
1/28	6.99	2.031	5/23	6.42	3-day embryo	10/18	4.80	2.115
2/5	7.13	2.141	5/29	6.16	*Hatched*	10/26	5.06	
2/13	6.48	2.080	6/5	5.92	Infertile	11/3	4.83	2.221
2/15	7.66	2.278	6/8	7.01	Br., thin	11/13	4.96	2.180
2/21	6.62	2.084	6/14	5.98	3-day embryo	11/23	4.88	2.355
2/28	6.44	2.201	6/27	5.18	Infertile	12/1	5.02	2.308
3/7	6.63	2.179	6/29	5.91	Trace devel.	12/8	4.87	2.214
3/9	6.96	2.101	7/8	6.26	2-day; thin	12/15	5.00	2.352
3/15	6.01	1.749	7/16	5.34	Abn. dev.	12/23	4.73	
3/21	6.68	2.191	7/23	4.95	2-day embryo	12/29	Th. shell, br.	
3/23	7.11	2.220	7/31	4.60	1-day embryo	12/31	5.72	2.321
3/29	6.70	2.018	8/2	5.48	2-day embryo			
3/31	7.27	2.237	8/11	5.00	2-day embryo	Ave.	6.01	2.170(59)
4/6	6.59	2.021	8/19	4.86	1-day embryo			
4/8	6.80	1.984	8/28	5.10	2-day embryo	(60 eggs) 30 singles		
4/14	6.26	1.857						

* Female A622 was dosed daily with calcium lactate and calcium lacto-phosphate from November 12 to December 20, 1919.

† The egg-albumen found to be qualitatively abnormal from September, 1920.

Details of data for case 1. A detailed presentation of the complete reproductive record of the five females would involve an excessive amount of tabular matter. The complete record for case 1 (♀ A622) during the period of her reproductive abnormality, together with typical data from her earlier period of normality, are given in tables 2 to 4. This supplies practically all of the details for the bird most intensively affected; it gives

TABLE 3

Complete record of ♀ A622 for 1921 (40 eggs). Various substances added daily to the normal diet in the amounts and during the periods indicated

DATE	WEIGHTS AND FERTILITY	RATE OF LOSS PER HOUR	DATE	WEIGHTS AND FERTILITY	RATE OF LOSS PER HOUR
		mgm.			*mgm.*
1/7	5.00 2.112			K_2HPO_4 0.170 g. (to 9/5)	
1/14	5.01 2.268		5/15	4.62 Thin shell	
1/20	4.72 Thin shell		5/21	4.79 Thin shell	(10.3)
1/22	5.46 2.220		5/23	5.17 0.5-day embryo	(9.3)
1/28	5.01 2.208		5/29	4.57 Thin shell	(23.6)
2/4	4.60 2-day embryo	(7.3)	6/4	4.52 Thin shell	
2/10	5.13 Thin shell	(14.0)	6/13	4.67 Thin shell	(14.5)
2/18	4.72 2-day embryo	(7.2)	6/20	4.51 Thin shell	
	Vit. B (yeast) 0.211 g. (to 5/2)			Milk powder, 0.513 g. (to 9/14)	
2/24	4.80 Infertile	(9.8)	6/26	4.22 Thin shell	
3/2	4.90 2-day embryo	(12.0)	6/29	5.23 0.5-day embryo	(11.0)
3/6	5.00 1-day embryo	(10.0)	7/7	4.36 Slight dev.	(16.3)
3/13	4.43 1-day embryo	(5.7)	7/9	4.87 Slight dev.	(13.0)
3/20	5.02 1-day embryo	(10.9)	7/15	4.57 1.948, thin*	
3/23	5.70 1-day embryo	(10.1)		*Thymus* 0.020 g. (8/3 to 1/22)	
	Vit. C (orange) 10 cc. (to 5/2)		8/25	8.78 Hatched	(3.2)
3/29	4.83 1-day embryo	(9.8)	8/27	8.39 Hatched	(2.6)
4/1	5.52 Thin shell	(27.3)	9/19	8.89 6-day embryo	(3.5)
4/7	4.96 0.5-day embryo	(10.6)	9/21	8.99 Hatched	(5.0)
4/9	Soft shell		9/29	8.79 2-day killed	(2.8)
4/16	5.18 Thin shell	(11.3)	10/1	(2.30) "Wind egg;" tr. yolk	
4/23	4.85 1-day embryo	(9.5)		New ♂ mates (3) given (to 12/7/21)	
5/1	4.95 Infertile				
5/2	Soft (premat.)				

26 clutches before *thymus* = 18 singles; 23 clutches after = 0 singles

* Air chamber double and movable in many eggs preceding this period. These not present in eggs following thymus dosage.

also the results obtained from two periods of thymus dosage when administered to a single bird. At the top of table 2 are given fragments of data obtained during the first three years of this bird's reproductive life. The first six eggs in life (early 1917), and others which followed were of normal size, provided with normal yolks, and hatched when incubated. The first six eggs of the following year (1918), and the later eggs of the same year,

were also entirely normal. During the early part of the following year (1919) also her reproduction was normal. At the end of that year, however, the table shows a decrease in the amount of egg-envelopes present, and two of the last eggs are singles. Three of the 39 eggs produced during this third reproductive year were singles; none of 30 eggs of the preceding year were singles; and none of the 20 of the first reproductive year were singles. If we pursue an examination of this point into the years following 1919 it will be seen that 30 of the 60 eggs of 1920 were singles; 18 of 34 eggs before thymus dosage in 1921 were singles. In 1922, 8 of 10 clutches preceding a second period of thymus dosage were singles.

At the bottom of table 2 is given the complete record for the fourth year of reproduction (1920). Yolk size remained normal, but the quantity of the egg-envelopes steadily decreased during the production of 60 eggs. From 21 tests of these eggs only one bird was hatched. Also, single eggs, thin shells, infertility, and early dying embyros appeared in profusion. During this year the egg "albumen" became quite abnormal. This fact was first noted through the observation that in eggs subjected to a steam bath (for the coagulation of egg contents preliminary to a determination of yolk weights) the small amount of albumen remained watery and uncoagulated. Further tests showed it was also not precipitated by saturated magnesium sulfate. Three albumen samples were thereafter painstakingly separated from eggs laid at this period for the purpose of nitrogen determination. An initial error of the assistant in starting these determinations, however, resulted in the loss of all these samples; this occurred when it was already too late to obtain other samples. The qualitative tests earlier made, however, indicate that the "albumen" being secreted at this period was probably an ovomucoid.

The record for the fifth year of reproduction (1921) shows that the egg-envelopes were reduced to an extraordinary degree in all of the 34 eggs laid before thymus dosage; yolk size remained essentially or absolutely normal. Hatches were impossible; eggs not infertile yielded only embryos that died early. Every egg was provided with a thin shell, as shown by its measured rate of (weight) loss or by simple inspection. Most eggs had movable and double air chambers. Various nutritional substances, including large amounts of the protein of milk, failed to have any effect on any of the above abnormalities. The administration of 20 mgm. of desiccated thymus was, however, promptly followed by a return to normal egg size (through increased production of albumen), to shells of normal thickness, and to eggs usually capable of hatching. With a different male mate the several eggs laid during and immediately after thymus dosage were all capable of hatching.

Table 4 shows that this first period of thymus dosage was discontinued on January 22, 1922. It was not until October 15 of the same year that

the amount of albumen had again been sufficiently reduced, and the number of "single" eggs so increased, as to warrant a second rigorous test of the effect of thymus administration in this bird. Even at this time nearly 2 grams more albumen per egg was present than when thymus was first administered 14 months earlier. The results show that again the size and

TABLE 4

Complete record of ♀ A622 for 1922

DATE	WEIGHTS AND FERTILITY	DATE	WEIGHTS AND FERTILITY	DATE	WEIGHTS AND FERTILITY
1/7	9.05 Hatched	4/26	8.27 2.013 In.	8/2	6.85 2.308 In.
1/9	9.46 Hatched	4/28	9.20 2.235 In.	8/10	6.97 2.272 In.
1/21	8.71 Hatched	5/4	8.49 Hatched	8/17	6.05 2.142 In.
1/23	8.82 Hatched	5/6	9.35 2.335 In.	8/28	6.79 2.392 In.
Last Thymus on 1/22		5/12	8.45 14-day embryo	9/5	6.24 2.155 In.
2/2	8.72 Hatched	5/14	9.36 5-day embryo	New ♂ mate on 9/6	
2/4	8.55 Hatched	New ♂ mate on 5/16		9/12	6.45 14-day embryo
2/12	8.70 Hatched	5/24	7.84 Hatched	9/18	6.61 7-day embryo
2/14	9.06 Hatched	5/26	8.77 Hatched	9/20	7.30 Hatched
2/22	8.86 Hatched	6/1	7.90 Hatched	9/28	6.55 2.335 In.
2/24	8.84 8-day embryo	6/3	8.56 Hatched	10/5	6.58 2-day embryo
3/4	8.43 Hatched	6/9	8.08 Hatched	10/14	6.61 Hatched
3/6	6.26 3-day embryo	6/11	8.36 Hatched	*Thymus* 0.020 g. daily	
3/14	8.32 Hatched	6/17	7.81 Hatched	10/29	8.32 Hatched
3/16	8.61 Hatched	6/19	8.32 Hatched	10/31	9.42 Hatched
3/23	8.72 Hatched	6/25	7.47 Hatched	11/10	9.06 Hatched
3/25	7.99 2-day embryo	6/27	8.33 3-day embryo	11/12	9.24 Hatched
4/1	8.37 Hatched	7/3	7.57 4-day embryo	11/26	9.25 2.287
4/3	8.88 Hatched	7/5	7.95 Hatched	Last Thymus on 11/27	
4/10	8.24 2.111 In.	7/12	7.10 Infertile	12/6	9.07 2.178
4/12	7.52 1.818 In.	7/24	7.00 2.269 In.	12/8*	9.97 2.525
4/18	8.31 1.967	7/26	8.07 2.571 In.		
4/20	9.09 Hatched				

Of 10 clutches before thymus 8 = single; of 4 after, 1 = single
Air cavity moved in many eggs laid after June

* ♀ A622 killed 12/8/22 at 2:15 p.m.—4 hours after laying second egg of clutch. Keel bone slightly crooked; pubic bones very wide = 17.2 mm. Liver normal or slightly enlarged (3.585 g.), with a few white flecks but no tubercles; spleen slightly enlarged (0.064 g.). Kidneys of normal size and appearance. Right ovary present (7 mm. long), its largest ovum, 1.5 mm. in diameter; no right oviduct. *Thymi* extremely small (R. = 0.0169 g.; L. = 0.0146 g.), preserved. No tuberculosis; 13 *Ascaridia*.

developmental capacity of the eggs promptly returned to the normal. That they really became normal is shown by comparing their size with the size of eggs produced by this same female during 1917 and 1918. The autopsy of this female, killed 4 hours after laying the egg last listed in table 4, is appended to that table.

Results of thymectomy. Attempts have been made to obtain from thymectomized reproducing female pigeons further evidence concerning the relation of the thymus to processes of reproduction. The principal conclusion we draw from the results of our attempts at thymectomy is that the getting of other facts than those now fairly clear is a difficult task in birds. We shall here record the results of our own efforts and then review them in the light of an important earlier observation.

Before thymectomy was attempted (beyond an exploratory operation in a living bird) two reproducing pigeons showing apparently normal reproduction were selected for the work and adequate records demonstrating this normality—particularly the thickness of their egg shells—were obtained before operation. One of these two birds was lost in the operation. The other bird survived the operation but died shortly afterward showing an unaccountable hemorrhage from the right auricle. In this second case it had been thought that total thymectomy was probably effected and our macroscopic examination after death failed to find thymic tissue. Later results, however, lead us to question the completeness of the operation.

Two additional female common pigeons and a female ring dove, for all of which we had less complete preliminary reproductive data, were next operated. The ring dove died at the conclusion of the operation. The thymectomy was thought to have been complete. Examination made after death, when the operated field was free of flowing blood, and was otherwise freed of blood stains, led us to question the totality of this extirpation. The two common pigeons survived what was thought to be "almost or quite complete extirpation." In both of these birds the wound healed without showing infection. One bird, however, lost weight (80 grams) and, though it had otherwise appeared in fair or good condition, died 19 days later without having produced eggs. Many *Ascaridia* were present and apparently these had punctured the intestinal wall and caused death. We think it significant that, notwithstanding the emaciation and the probable peritonitis preceding death, a plain trace of the right thymus was found.

The other thymectomized female later produced eggs. The complete reproductive record of this female (V243) is given in table 5. This bird had become sexually mature earlier (first egg at 5.5 mo.) than the average pigeon, and laid freely. Only 8 eggs were laid previous to thymectomy (at 6.8 mo.); and beginning 15 days after the operation she continued to lay eggs in fairly rapid succession at intervals of eight to ten days between clutches. Under these circumstances we must expect, as the writer has earlier shown, a gradual *increase* in yolk size and total egg size. It will be seen that some slight increase in total egg size did occur after thymectomy,

TABLE 5

Reproduction record of a thymectomized common pigeon (♀ V 243)

DATA ON EGGS			DEVELOPMENT	DATA FOR SHELLS		REMARKS
Number	Date	Weight (grams)		Rate of loss (mgm. per hour)		
A1	1/21	(First egg in life; age of ♀ = 5.5 mo.)				
A2	1/23	Not weighed	Not tested	Good		
B1	2/2	Not weighed	Not tested	Good		
B2	2/4	Not weighed	Not tested	Good		
C1	2/14	Not weighed	Not tested	Good		
C2	2/16	15.82	Not tested	Good		
D1	2/24	16.24	Hatched	4.4		
D2	2/26	16.08	Injured embryo	6.1		
Thymectomy on 3/3 "almost or quite complete"					Ave. = 5.1	
E1	3/18	16.02	1-day embryo	4.1		
E2	3/20	16.90	Infertile	5.6		
F1	3/28	16.57	Hatched			
F2	3/30	16.75	Infertile	9.4		
G1	4/7	16.57	Infertile	7.5		
G2	4/9	16.52	Infertile	6.3		
H1	4/17	16.68	Broken			
H2	4/19	16.99	Broken		Ave. = 6.9	
I1	4/27	17.60	4-day embryo	5.8		
I2	4/29	16.33	Infertile	8.3		
J1	5/7	17.39	Hatched	4.8		
J2	5/9	16.04	Infertile	5.9		(Air cavity moves)
K1	5/20	17.12	Hatched			
K2	5/22	17.28	Hatched	4.8		
L1	5/30	17.62	5.5-day embryo	3.8		
L2	6/1	17.42	Hatched			
M	6/10	16.23	Infertile	3.0		
N1	6/19	16.17	Hatched	4.2	Ave. = 4.2	
N2	6/21	16.39	7-day embryo	4.2		
O1	6/29	16.75	3-day embryo	5.0		
O2	7/1	16.95	Hatched			
P1	7/9	16.69	3.5-day embryo	4.0		
P2	7/11	17.08	Hatched			
Q1	7/19	17.22	Hatched			
Q2	7/21	17.71	Hatched			
R1	8/1	16.98	Hatched			
R2	8/3	16.27	15-day embryo			
S1	8/12	16.93	5-day embryo			
S2	8/14	17.33	Hatched			
T1	8/23	16.65	Hatched			
T2	8/25	17.07	Hatched			

Male mate became emaciated; removed 9/20, and new mate given, but no eggs.

Heart-punctures 10/27 and 10/28 on ♀V243 for blood-sugar samples (sugar = 0.160 per cent).

♀V243 killed for examination 10/31. Weight = 311 g. Healthy, but ovary very small (minus a single ovum of 5.5 mm. diameter = 0.0065 g.). Thymus: 4 isolated traces on right side (0.0133 g.) and 3 similar traces on left side (0.0168 g.); total, 0.0301 g.

but whether the full amount of the expected increase occurred is a question that can not be answered. It is clear, however, that no decrease in albumen secretion became evident here.

A rather high percentage of infertility was found during two months following the operation, and two early-dying embryos also belong to this period. These observations lose something of definiteness, however, because of the incompleteness of the fertility record in the pre-operation period.

In view of Soli's results (discussed below) on the appearance of thin shells following thymectomy in reproducing fowls, we were particularly interested in the possible effects of the operation on the shell thickness in the case of the pigeon. It was later found that the pressure of other work had unfortunately caused us to overlook the determination of shell thickness in a few instances and broken eggs prevented such determination in another two cases. It is not certainly known whether the two broken eggs indicate that these eggs had exceptionally thin shells. In three of Soli's four tests normal shells were not produced until the eighteenth or twentieth day following thymectomy. The two eggs laid by the pigeon on the fifteenth and seventeenth days may therefore be classified separately from the later eggs, or be grouped with the eggs belonging to the pre-operation period. These four eggs indicate a shell thickness corresponding to an average rate of loss of 5.1 mgm. per hour. During the following 60-day interval this rate of loss averaged 6.9 mgm. per hour—demonstrating that eggs with thinner shells were produced at this time. In the last egg (May 9) of this period a "movable air cavity" was found, but this was the only noted instance of this defect. During the succeeding 50-day period the shell thickness was increased so as to give an average loss of only 4.2 mgm. This means that at this period normal or unusually thick shells were produced.

To the bottom of table 5 other data are added. The condition of the thymus, as found when the bird was killed particularly for its examination, requires some comment here. At the time of operation we had thought the thymectomy "almost or quite complete." The final examination nevertheless made it certain that we had left traces of thymic tissue in at least seven isolated places. We feel fully justified in stating that the size of some of these fragments was unquestionably larger than any portion that could have been overlooked at the time of operation; and that the *regeneration* of thymus tissue assuredly occurred in the case of this pigeon. On the other hand, extensive observation on the speedy and sometimes complete involution of the pigeon thymus in disease gives equal assurance that if we had waited to make this final examination until the bird became diseased (as Soli possibly did) we should then have found a smaller thymus or no thymus in this bird. It is further by no means certain that we exam-

ined this bird at the time it possessed the greatest amount of regenerated thymus. The thymic tissue recovered weighed 30.1 mgm. This was healthy well-rounded tissue, occurring as isolated nodules, and probably contained a minimum of connective tissue. If we calculate the "expected" or normal size of the thymus in a common pigeon of her age, sex, etc., in the same way this was done for two other groups above, a value of 214 mgm. is found.

The data obtained in the five thymectomies, though few in number, go far toward convincing the writer that complete thymectomy in adult pigeons is a difficult thing to accomplish. Our own results, in common with other work to be cited in the following section, further show that if minute fragments of the bird's thymus escape removal these regenerate to a marked degree. It follows that the results recorded in table 5, and in Soli's experiments as translated and put in tabular form by us (table 6), must be viewed in the light of these two important considerations.

Soli's (6) four operated birds produced eggs with apparently normal shells (tested only by inspection) until two to three weeks after removal of the thymus. In his material he could not or did not distinguish the eggs, if any, which were laid prematurely and were thus necessarily deficient in shell material. In his "first case," however, the egg weights are recorded and these data indicate that only the egg of July 17 (the very first shell-less egg of the series) was without shell and undersized for this reason. If this is true the four birds all began producing obviously thin or shell-less eggs at 17 to 20 days after thymectomy. We think that the uniformity found in this matter by Soli is a point of much significance. The restitution of shell material began at 28, 30, 39 and 45 days after the operation. It is stated definitely that the fowl of the "first case" continued to lay, except for normal rest periods, during two years and that when killed and examined for thymus none was found. From the history given, and the considerations mentioned above, it is quite probable that thymus tissue had regenerated in this fowl and that the bird was killed only after its involution had taken place. The amount of albumen secreted on the eggs which followed the operation was apparently slightly reduced—since eggs entirely without shell lose only 5 or 6 grams in weight from this source—but certainly no very marked reduction occurred. This also essentially duplicates the results obtained for the pigeon—in which a regeneration of the thymus was demonstrated.

Discussion. In the numerous previous studies involving thymectomy it appears that only Soli had observed the effects of this operation in adult reproducing female animals which secrete egg-envelopes. It thus happens that this work only has a direct bearing upon the particular function of the thymus under consideration here. Soli's experiments, however, were performed as a test of the relation of the thymus to the calcium metabolism,

and his observations and conclusions were thus given a restricted application. Soli concluded *a*, that thymectomy causes a notable modification of the calcium metabolism, this leading to a total suppression of the calcium laid down in the shell of the fowl's egg; *b*, that this probably results from a decreased absorption of calcium by the intestine and a decreased utilization of the blood calcium by the tissues; *c*, that these effects are

TABLE 6

A tabulation of data obtained by Soli on thymectomized fowls

DATE	WEIGHT OF EGG	SHELL	OTHER CASES
First case			Third case
6/29	65.6	Good	After operation, "normal shells to 20th day"
7/1	63.7	Good	
Thymectomy "complete" on 7/3			20th day "a very fragile shell"
7/5	60.6	Good	23rd day, no shell
7/7	59.9	Good	10 other "no shells" to 45th day
7/8	63.1	Good	45th day "began laying normal shells"
7/11	59.3	Good	Fourth case
7/13	55.9	Good	For 16 days eggs with normal shells
7/15	60.0	Good	On 18th day, no shell
7/17	50.0	None	During next 20 days 9 eggs with no shell
7/19	62.1	With shell	At 39 days began laying normal eggs
7/20	54.0	None	Fifth case
7/21	54.2	None	Control cutting—thymus not removed
7/25	59.0	None	In 2 months "produced no bad shells"
7/28		None	Sixth case
7/29	Broken	None	Control cutting—taking out only two posterior lobes of thymus
7/31	59.0	Thin	In 2 months "eggs showed no appreciable alteration" in shells
8/2		Less thin	
8/4 (and later) "apparently normal"			

Second case

First 16 days after operation = "Normal eggs"
18th day = "fragile—calcareous scales only"
To 23rd day, two eggs like that of 18th day
Later, 1 with thin uniform calcification
Next, 2 "without shell" (membrane only)
After 30th day, eggs with imperfect shells
On 40th day, began to lay eggs "apparenty normal"

never produced immediately, but 15–20 days after the operation, and that still later a complete recovery gradually ensues; *d*, that neither the trauma involved in the operation nor partial thymus extirpation causes any of these disturbances.

It is obviously both impossible and unnecessary to attempt a full consideration of the large amount of work that has been done on the function of the thymus. We have of course tried to examine the entire literature

of the subject in order to check our own results with those of others. Park and McClure (7), besides conducting a careful study, have contributed a most valuable examination and review of the earlier work in this field; and Hammar (8), who has contributed so largely to all aspects of our knowledge of the thymus, has recently published an extensive review of the literature. In our opinion this earlier work provides considerable evidence that the thymus is in some way related to the calcium metabolism, and also to the reproductive organs, in at least several of the animals studied. These relationships have been observed even in some mammals and it is obvious that the particular function of the thymus disclosed here would probably imply both of the above-mentioned functions as correlative or associated aspects of this primary or original function—the control of the secretion of the egg-envelops. The eggs and oviducts are themselves essential parts of these reproductive organs, and a special *calcium gland* is located in the oviduct or uterus. Our observations seem to have no bearing on the lymphoid or antitoxic functions of the thymus except that in so far as the present data suggest a primary function of the thymus other functions are thus indicated as probably of secondary origin.

As a further statement on the present state of our knowledge of the physiology of the thymus we may quote at this point a summary recently written in *Endocrinology and Metabolism* by Hoskins (9): "The literature as a whole affords little or no reliable evidence that the thymus has any true endocrin function. In all probability the organ is of significance in the physiological and pathological processes merely by virtue of its lymphoid character. Whatever function it has probably is concerned with the defensive mechanism against infections."

The practicability of total thymectomy and the regenerative capacity of the thymus require consideration. Hammar (10) proved by subsequent serial sections of the entire neck region that the thymus could be completely removed from frogs; that in many of these cases no restitution occurred; and that thymus residues left at operation did not hypertrophy. In this work Hammar also proved that the thymus is not essential to the life of the (we must add, of the *individual*) frog. It may be mentioned, however, that Aimè (11) later noted that in turtles the thymus normally reaches its maximum development in the autumn and that it thereafter temporarily involutes in winter. Though Hammar's extirpations in the frog included both a spring and an autumn period it is not impossible that this work failed to cover the most favorable season for regeneration. The period between operation and examination of these frogs was 12 to 60 days. Park and McClure think it difficult to remove all traces of thymus in mammals but they apparently demonstrate that the thymus has little capacity to regenerate in dogs. Fulci (12) and other investigators have reported a marked regeneration of the thymus in rabbits, dogs and cats.

In the case of birds, however, it was clearly shown in the only previous work in which these points were properly examined that it is an extremely difficult matter to extirpate completely the elongate double thymus; and also evident that much and fairly rapid regeneration does occur in these animals. Basch (13) took all the thymus visible with a lens from two doves four weeks old. After 6 and 8 weeks these doves were killed and are reported as having nearly as much thymus tissue as their controls (no weights given). From five very young chicks, killed about three months after the operation, he got similar results. In our own experience we have found it an exceedingly difficult matter to remove all traces of the dove thymus; and we have obtained unmistakable evidence of its regeneration.

We attach importance to the observation that there is a delay of 14 to 20 days in the onset of functional disturbance following thymectomy in various animals. Basch (according to Soli) was first to report that the effects are delayed; the point studied in this instance being the increased calcium excretion in the urine. Klose and Vogt (14) reported a latent period of about 14 days, though Park and McClure have pointed out objections to the full acceptance of their results. Soli obtained wholly consistent evidence of a delay of 17 to 20 days in birds. Our own thymectomized dove conforms to the results of Soli on the fowl. Quite similarly we must interpret our experience with thymus feeding of the five doves which developed thymus deficiency, since the minute amounts fed were able to exercise a marked influence during a considerable period after discontinuance of dosage in animals practically deprived of thymus. The response of these animals to thymus administration was immediate, but the specific effects of dosage were notably prolonged. Probably it is on this same basis that we must interpret the paradoxical continuance of thymus function for two or three weeks after its removal. Even after that lapse of time the effect was upon the calcium gland chiefly, and the albumen secreting function was able to continue for an even longer though unmeasured period. It seems probable that this capacity of this thymic product to resist all but slow destruction in the blood is reflected in our observation on its effectiveness, and therefore its integrity, after subjection to the action of the digestive enzymes. Is this capacity to be looked upon as a safeguard to the eggs—that is, to the continuance of the species—against an incretory organ highly susceptible to temporary involution from a variety of causes?

The somewhat unusual and paradoxical nature of this substance is fairly evident. That it is a product definitely associated with the thymus, and that it acts specifically and immediately to restore the secretion of the egg-envelopes in a group of essentially thymoprivous birds, seem to have been demonstrated. It seems also to have been established that these effects tend to persist after a last administration; and that similarly

the normal action of the thymus also tends to persist for an unexpected period after its removal. This unusually prolonged or continued action of the thymic product after removal of the thymus, and the capacity of minute quantities of the substance given orally to remove the effects of thymopriva, perhaps also assist the comprehension of the following situation as stated by Hammar (8): "There is no morphological observation that could justify the idea that the thymus carries out any secretory activity in the real meaning of this term." Obviously, neither an approximately continuous, nor at any time more than a diminutive, secretion would be required to fulfill completely this primary function of the gland. Only in certain restricted stages of the reproductive life of the female is this substance thus required. It therefore seems that decisive morphological evidence could only be found in suitable species (not mammalian) taken at appropriate stages and examined in the light of facts now available.

Further tests on this function of the thymus can of course be made in suitable vertebrate species by means of thymectomy properly examined and controlled. It is now clear that the animal used must be an egg-laying vertebrate—preferably a bird or reptile. While it is true that the relation of the thymus to the production of egg-envelopes has been shown only in birds (two orders) the homology of these structures in the lower and higher vertebrates is unquestioned, and it may be assumed that the mechanism governing egg-envelope production is a similar one throughout the vertebrate group. If a bird is used in such study, a second operation following the initial regeneration of isolated nodules of tissue, will be required to effect a complete removal. Moreover, any test of the completeness of the removal will be quite inconclusive unless this examination is carried out in healthy well-nourished animals—if possible, in cases where these conditions are made evident by a continuance of ovulation in addition to the findings at necropsy.

Once a supply of reproducing thymus-free animals is available for further tests it should be a simple matter to identify, more or less definitely, the active thymic substance. Additional work of this and other kinds is obviously necessary. It is evident that rather more questions are raised than are resolved by the present investigation. The pressure of other work prevents, for the present, any adequate examination of many interesting aspects of the problem in our own laboratory. The physiology of reproduction rather than the physiology of the thymus formed the point of ingress to the study described here; and reproduction is being studied by us chiefly because of the contribution of such studies to our main problem—the nature of sexuality. Our own plans for the further examination of the present subject include only the further testing of additional birds which may show or develop this unfortunately rare type of abnormality, together with an attempt—now in progress—to bring about experimentally an

early involution of the thymus. If successful in this attempt we hope to record the syndrome of resulting abnormalities, and in these cases make further tests of the restorative action of various chemical fractions of thymus tissue.

We do not overlook the fact that the substance of thymic origin considered here has not been given the special type of examination and test which physiologists have considered necessary to a demonstration of a true hormone. In the light of the unusual situation developed in the case of this substance—one not primarily concerned with the individual life but with that of the species—there is found, however, some reason for a full consideration of somewhat different tests. We are unable to interpret consistently the foregoing facts on any basis which does not regard this substance as an internal secretion of the thymus, and one which is not quickly or easily destroyed in the body. It would seem convenient to have a name for this imperfectly identified substance, and perhaps appropriate that this name indicate both its place of origin and the point of its primary action (the oviduct). The term *thymovidin* is suggested.

The results of the present study make it easy to account for the relatively meagre positive results, and the often conflicting reports, that have attended the numerous studies on thymus function in mammals. If the primary function has been lost in these forms along with the great transformation of their reproductive processes—involving a complete loss of the egg-envelopes—and with the origin of new means of chemical regulation (corpus luteum, etc.) of the newly developed mammalian type of reproduction, it becomes practically impossible to discover the primary function of the thymus in this group of animals. On the other hand, the studies that have hitherto been made on lower vertebrate forms have unfortunately failed to test its relation to reproduction. Its relation to continued life, growth and similar points have been sought rather consistently on young instead of adult individuals. Using thymectomy as the method of study only Soli has looked in proper material for any effect on any aspect of reproduction. In that case, a strong impression of the relation of the thymus to the calcium metabolism, a probably incomplete extirpation followed by unsuspected thymus regeneration, and a probably too short thymoprivic interval, united to prevent the disclosure of the highly important reproductive rôle of this organ in vertebrates other than mammals.

The foregoing data further conform to previous experience in indicating that the thymus is in no way essential to the life of the individual; though, equally important with that point, it does appear to be and to have been quite essential to the continued existence of vertebrate groups other than mammals. There are now moreover no data available which exclude the possibility that at least an early stage of thymus development is entirely essential to the proper development, and therefore to the later function-

ing, of the mammalian uterus. And mammals themselves probably could not have come into existence without the earlier presence of the thymus, since this organ governed the building of the egg-envelopes within which the embryos and young of mammalian ancestors were protected until capable of an independent life. This species-preserving aspect of thymus function as disclosed here obviously suggests that in this case we are probably dealing with the original or primary function of the gland—a gland which heretofore has sometimes been called the "enigmatic organ."

SUMMARY

A prolonged study of the etiology of several types of reproductive abnormalities in pigeons has resulted in the isolation of one type of disorder which is readily corrected by the oral administration of ox thymus.

This particular type or syndrome involves: Eggs with yolks of normal size but deficient in shell and albumen; frequent reduction of normally paired ovulations to single ovulations; diminished fertility and a restricted hatchability of eggs. In addition, birds showing these abnormalities initially had shown quite normal reproduction. All of the five birds showing these abnormalities were found at necropsy to have extremely small thymi.

Only birds of the particular type described were affected by thymus administration. Dosage with other substances, including a few tests of other desiccated tissues, had no effect on this type of abnormality.

Complete thymectomy is difficult to obtain in pigeons and there are many sources of error in evaluating the completeness and the results of the operation. From one partially successful test data were obtained in conformity with Soli's, more extensive but inadequately interpreted results with thymectomized fowl.

The whole of the data seems to demonstrate the presence in the thymus of a substance having a highly specific action on the oviduct of birds—and presumably, of all those vertebrate animals which secrete egg-envelopes. The substance is indispensable to the production of normal egg-envelopes. It is apparently of the nature of a true hormone. To characterize it with reference to its place of origin and the principal point of its action the name *thymovidin* is suggested.

Several facts indicate that thymovidin is only very slowly destroyed in either the blood or the digestive fluids; but no step in its isolation from thymic tissue has been attempted.

Though not necessary to the life of the individual thymovidin would seem to be essential to the perpetuation of those vertebrate species whose eggs are protected by egg-envelopes. Such animals were the ancestors of mammals and thus mammals also probably could not have come into existence without the thymus.

BIBLIOGRAPHY

(1) RIDDLE: This Journal, 1921, lvii, 250.
(2) RIDDLE AND HANKE: This Journal, 1921, lvii, 264.
(3) JOLLY AND LEVIN: Compt. rend. Soc. de biol., 1911, lxxi, 374.
(4) JOLLY AND LEVIN: Compt. rend. Soc. de biol., 1912, lxxii, 146.
(5) MCCARRISON: Indian Journ. Med. Res., 1919, vi, 557.
(6) SOLI: Pathologica, Revista quindicinale (Genova), 1911, iii, 118.
(7) PARK AND MCCLURE: Amer. Journ. Dis. Child., 1919, xviii, 317.
(8) HAMMAR: Endocrinol., 1921, v, 543, 731.
(9) HOSKINS: Endocrinology and metabolism. New York, 1922, ii, 378.
(10) HAMMAR: Pflüger's Arch., 1905, cx, 337.
(11) AIMÉ: Compt. rend. Soc. de biol., 1912, lxxii, 889.
(12) FULCI: Deutsch. med. Wochenschr., 1913, xxxix, 1776.
(13) BASCH: Monatschr. f. Kinderheilk., 1907, vii, 541.
(14) KLOSE AND VOGT: Beitr. z. klin. Chir., 1910, lxix, 1.

CARDIO-VASCULAR REACTIONS TO HEMORRHAGE AND TRANSFUSION IN MAN

J. A. E. EYSTER AND W. S. MIDDLETON

From the Department of Clinical Medicine, University of Wisconsin

Received for publication March 3, 1924

Meek and Eyster have shown that hemorrhage (1) and transfusion (2), comprising an amount of blood within approximately 2 per cent of the weight, cause in the dog only transient effects on arterial and venous pressures, diastolic heart size and cardiac output. Within a brief period compensatory readjustments occur restoring normal circulatory conditions. Opportunities for extending these studies to man have been taken advantage of in the case of donors and recipients in transfusion procedures carried out in the University Hospital.

Methods. Diastolic silhouette area was determined by the use of a bedside x-ray unit. Exposures were made in the dorsal prone position with a distance of target to film of 1 meter or more, correction for distortion being made in each case. Venous pressure determinations were made with the apparatus of Hooker and Eyster (3). Systolic and diastolic arterial pressures and pulse rate were also recorded.

Effect of hemorrhage. Six donors were studied. The extent of bleeding varied between 0.62 and 0.83 per cent of the body weight. On the assumption that in man the total blood volume comprises about 8 per cent of the body weight (4), these amounts correspond to between approximately 8 and 10 per cent of the total blood volume. The period of bleeding was between six and ten minutes. There was a slight reduction in cardiac area in five of six cases shortly after the bleeding. The least reduction in the five cases was 2 per cent, the greatest 6 per cent of the silhouette area before bleeding. Corresponding reductions occurred in the transverse diameter. While changes of this magnitude are usually regarded as within the errors of teleradiogram technique, the constancy of the result seems to us significant. In all cases area and transverse diameter had returned to within 2 per cent of the normal an hour or less after the hemorrhage. There was usually a rather sharp fall in both systolic and diastolic pressures and a decrease in pulse pressure during and immediately after the bleeding but returning rapidly to the approximate normal. In most cases venous pressure also fell temporarily. Pulse rate changes were very inconstant and apparently

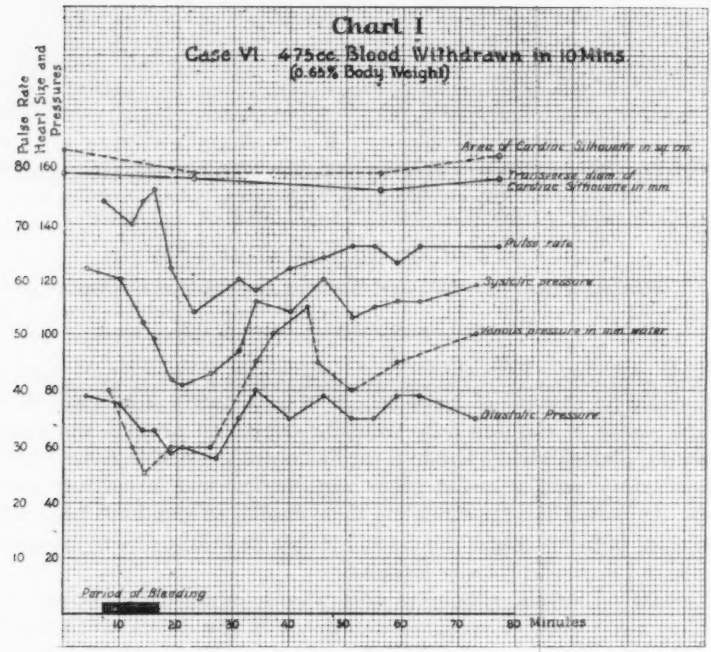


Chart I
Case VI. 475cc. Blood Withdrawn in 10 Mins.
(0.65% Body Weight)
Pulse Rate
Heart Size and Pressures
Area of Cardiac Silhouette in sq. cm.
Transverse diam. of Cardiac Silhouette in mm.
Pulse rate
Systolic pressure
Venous pressure in mm. water
Diastolic Pressure
Period of Bleeding
Minutes

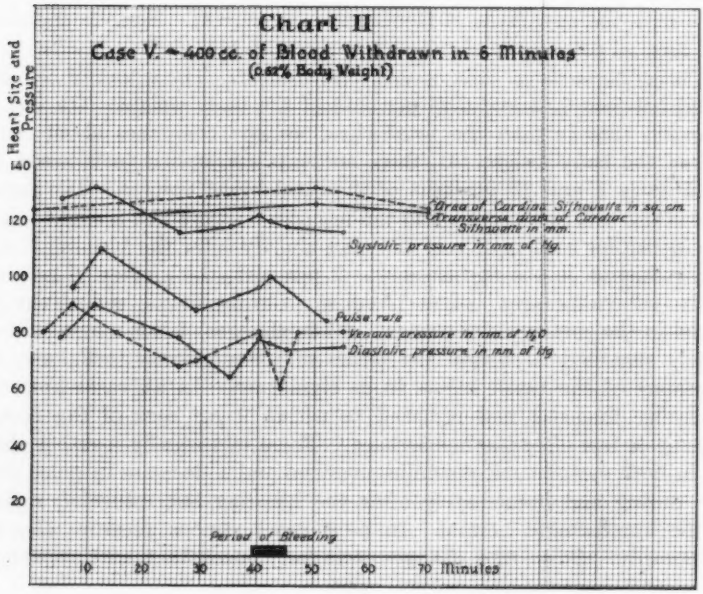
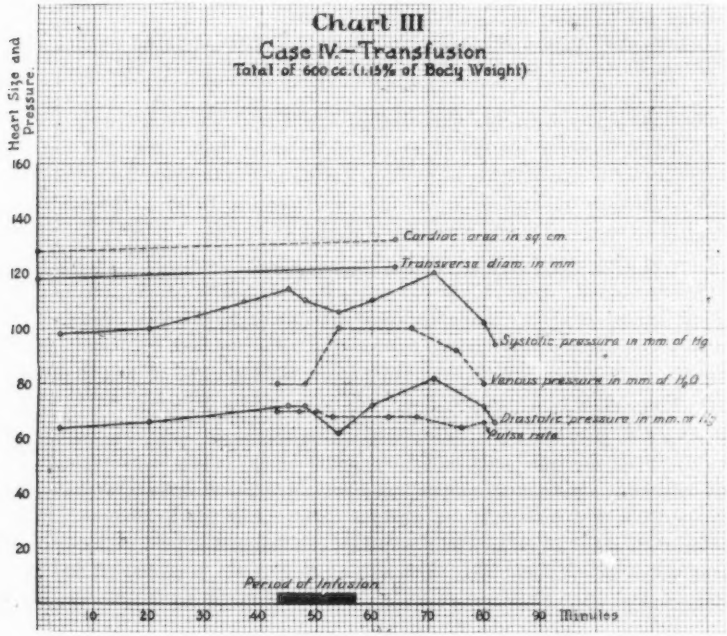


Chart II
Case V. - 400 cc. of Blood Withdrawn in 6 Minutes
(0.52% Body Weight)
Heart Size and Pressure
Area of Cardiac Silhouette in sq. cm.
Transverse diam. of Cardiac Silhouette in mm.
Systolic pressure in mm. of Hg.
Pulse rate
Venous pressure in mm. of H2O
Diastolic pressure in mm. of Hg
Period of Bleeding
Minutes

without significance. The greatest and least changes encountered in the six determinations are given in charts I and II respectively. The curves in chart I are also interesting in showing an early rebound in blood pressures with a smaller secondary fall. This was also noted in one other case.

Effect of infusion. Four recipients were studied during the process of infusion. The amount of blood infused varied between 1.00 and 1.15 per cent of the body weight. All were cases of primary anemia. The



period of infusion was between ten and fourteen minutes. Heart size changes were insignificant. Two showed a slight increase, one was unchanged, one showed a slight decrease in size. The maximum change was within 3 per cent in area and 2 per cent in transverse diameter. A transitory slight rise in systolic and diastolic pressures and in venous pressures was noted in all four cases during and immediately subsequent to the infusion. Restoration to normal occurred promptly. The case showing the most marked change is presented in chart III.

CONCLUSION

Hemorrhage and transfusion of blood in man, in amounts within 1 per cent of the body weight, result in only transitory alterations of cardiac size and blood pressures. Compensatory mechanisms cause a rapid readjustment to normal circulatory conditions notwithstanding the altered blood volume.

BIBLIOGRAPHY

(1) MEEK AND EYSTER: This Journal, 1921, lvi, 1.
(2) MEEK AND EYSTER: This Journal, 1922, lxi, 186.
(3) HOOKER AND EYSTER: Johns Hopkins Hosp. Bull., 1908, xix, 274.
(4) KEITH, ROWNTREE AND GERAGHTY: Arch. Int. Med., 1915, xvi, 547.

STUDY OF THE MECHANISM OF CHANGE IN RESISTANCE OF ERYTHROCYTES TO HYPOTONIC SALT SOLUTION

III. A Study of the Cause of Effects Produced by Cations on the Resistance of Red Corpuscles Previously Described

WINIFRED ASHBY

From the Division of Medicine, Mayo Clinic, Rochester, Minnesota

Received for publication March 10, 1924

In a previous study (1) it was found that human blood corpuscles incubated for three days in isotonic solutions of the chlorids of sodium or lithium, on a subsequent test with a scale of hypotonic salt solutions proved to be increased in resistance, while if the rubidium or potassium ion is substituted for the sodium or lithium ion, a marked decrease in resistance takes place. The result obtained by either of these two sets of ions can be reversed by the other, and the further the change has proceeded, the more readily the reversal can be accomplished. It was found that, in general, sodium and potassium bear the same relationship to resistance changes when in conjunction with other anions, the noticeable exception being in the cases of the citrate ion, both sodium and potassium citrate causing an increase in resistance. Incubation of corpuscles for two days in the chlorids of the bivalent metals, magnesium, calcium, strontium and barium, produced a marked increase in resistance with, at the same time, in the instances of the three latter metals, considerable cell destruction in the incubating fluid, as indicated by hemolysis. The increase in resistance produced by calcium, strontium and barium was not reversible by means of potassium. The purpose of work here reported was to investigate in the case of those metals for which a technic for quantitative determination is easily available, the mechanism by which these changes in resistance have been produced.

The first point of interest was whether or not these metals had entered the corpuscle and produced their effects by affecting the interior substance, or, without entering, had produced these changes in resistance by changing the state of the corpuscle at its periphery. Data have already been given indicating that the latter was not the case, and that changes in resistance were brought about either by loss of potassium or by the taking up of calcium; but more conclusive evidence seemed desirable.

The present status of the question of the relation of corpuscles to cations has recently been defined in a paper by Van Slyke, Wu and McLean,

who say: "The cell membranes are impermeable to the proteins, ionized or not, and to K and Na." They quote as authority Gürber, one of the earlier investigators, who worked with horse blood, and Doisy and Eaton, who recently have also used horse blood. Some recent work of Pick, however, is at variance with this point of view. He reports an effect on the perfused heart of the frog by the corpuscles of man and rabbit, which would indicate a release of potassium from corpuscles. He controlled his results with cat corpuscles which, unlike the corpuscles of man and rabbit, have an extremely low potassium content. Hamburger and his school have emphasized the relativity of the impermeability of the cell to cations. In a paper with Bubanovic, Hamburger offers evidence opposed to that subjected by Gürber, Hoeber and Okr-Blom who claim complete impermeability. Hamburger and Bubanovic, after treatment of beef corpuscles for three hours with a 5 per cent volume of carbon dioxid, added salt and water to the serum in amounts that were within physiologic limits. The results of chemical analysis indicated that, when water is added, sodium passes into the corpuscles and magnesium, calcium and potassium pass out, while, on the addition of sodium chlorid, there is a passage of sodium magnesium and calcium into the corpuscles, and of potassium out. How significant an exchange of cations between the corpuscles and their environment may take place, probably depends on several factors, such as the condition of the corpuscles, their degree of exposure to the cation in question, and the kind of corpuscles used.

In the present work human corpuscles have been employed; they have been exposed to straight isotonic[1] solutions of salts of the metals whose effects were being tested, for from two to three days, which of course is a severe and unphysiologic exposure. In the course of that exposure they underwent a progressive change which, although leaving them in most respects normal, was evidenced by a greater ease in production of the change in resistance typically caused by the metal in question. Whether or not this modification of the corpuscle ever takes place to any extent physiologically before the corpuscles cease to function in the circulation would be difficult to determine, although I suspect that such may be the case. But the point of interest here is that, as a result of this modification, marked changes in the cation content can be caused to take place, which are correlated with changes in resistance of the corpuscles to hypotonic salt solution, and which enable the mechanism of this change in resistance to be studied. In the course of this work data have also been obtained on change in corpuscle volume simultaneoulsy occurring with change in metal content, the the significance of which, with respect to the interpretation of change in resistance, is as yet a little doubtful.

[1] In this series of papers "isotonic solution" is used to indicate one capable of exerting an osmotic pressure equal to that of 0.9 per cent sodium chlorid.

TECHNIC. Approximately 100 cc. volumes of isotonic solutions of the salt or the mixtures of the salts, whose effects were to be tested were adjusted after sterilization to pH 7.3 to 7.5. The original solutions were made by adding 9.0 grams sodium chlorid, 11.5 grams potassium chlorid, 21 grams magnesium chlorid ($MgCl_2 \cdot 6H_2O$) and 15 grams of calcium chloride ($CaCl_2 \cdot 2H_2O$) respectively to a liter of water. They were assumed to contain 354 mgm. of sodium, 600 mgm. of potassium, 250 mgm. of magnesium and 416 mgm. of calcium respectively for each 100 cc. Amounts of citrated or defibrinated blood were added, ranging from 5 to 10 cc., depending on how much blood was available. In each series, the members of which are compared, the same blood measured by the same pipette was used. The incubator was run at 36°C. The bottles containing the blood were rotated on each day of incubation, except the last, to resuspend the corpuscles. On the last day, after note had been made as to hemolysis, the supernatant fluid was removed and the settled corpuscles were washed into a 15 cc. centrifuge tube with some of the salt solution used as the medium. In the absence of hemolysis, the corpuscles were considered good for volume data. Corpuscles in suspension in the supernatant fluid were recovered and added. The whole was centrifuged for half an hour at the third speed of a four arm Victor centrifuge, and the volume after sedimentation noted. After withdrawal of the supernatant fluid by means of a capillary pipette, so that only a very small amount of fluid comprising the miniscus remained, a known amount of corpuscles was removed for the fragility test. The remaining corpuscles were washed into a 50 cc. flask with distilled water for quantitative determination of the metal in question.

As hemolysis with water was difficult to accomplish in the instances in which resistance had increased, 1cc. of 10 per cent concentrated hydrochloric acid was routinely added before precipitation with trichloracetic acid; otherwise, except that the corpuscles were hemolyzed in the absence of serum, the technic recommended by Kramer and Tisdall for the determination of potassium, sodium and calcium in whole blood was used. For the technic used in determining magnesium I am indebted to Mr. L. B. Ritter of the clinical laboratories of the Mayo Clinic. It is a greatly simplified one which uses the well known precipitation of magnesium as a phosphate and subsequently the principle employed by Denis, that of estimating magnesium by its phosphate equivalent. The strychnine molybdate reagent and color development as given by Tisdall for the determination of phosphate in blood were used for the determination of the phosphate equivalent.

Resistance tests were run with hypotonic salt solutions which contained the citrate equivalent of 0.06 per cent salt. This acted as a buffer to maintain a more constant hydrogen-ion concentration. Two hundredths of

a cubic centimeter of corpuscles were added to 1 cc. of hypotonic solution. The readings were made on the bases of 0 to 4, 4 being complete hemolysis.

By the methods of Kramer and Tisdall for the determination of calcium and potassium an appreciable error is introduced by overwashing the precipitates. In view of this, it is evident that if there should be a much smaller amount of either of these elements in the test substance than is present in the blood and serum samples for which the test was designed, the results obtained would be too low. Since the changes which I am studying are great, and the degree of difference which takes place in the corpuscle cation content meets the demands of this investigation fully as well as a more absolute determination of the content, no attempt was made to overcome this error. The metal determinations as given in the protocols are calculated on the basis of the corpuscle mass after a half-hour centrifuging.

Results. In order to provide a variation in the mode of attack that might bring to light unexpected factors, and also to provide against the factors of deterioration of the corpuscles and of the effect of slight difference in isotonic value of the solutions on the volume changes of the corpuscles, in the work with the sodium and potassium salts, blood was incubated for a different length of time in each of these salt solutions, and for the same length of time in different mixtures of them. The effects of magnesium and calcium chlorids on changes in resistance were studied after uniform incubation in the isotonic solutions of the respective salts diluted to varying extents with isotonic potassium chloride solution and with isotonic sodium chloride solution. A preliminary test of the effect of washing without incubation was made.

The cation and volume changes resulting from washing. *Protocol 1.* For the change in volume determinations equal amounts of blood were compared. These were washed with isotonic solutions of magnesium, sodium and potassium chlorids, respectively, and centrifuged simultaneously with the control blood for half an hour at a high speed. After centrifuging, the volumes were read and the supernatant fluid was removed finishing the process of removal with a capillary pipette (table 1).

The amounts of metals in the solutions used for washing were calculated to be: potassium, 600 mgm. for each 100 cc.; magnesium, 250 mgm. for each 100 cc.; sodium, 354 mgm.; calcium 83 mgm. for each 100 cc. The difference between the sodium content of the unwashed corpuscles and of those washed with the sodium chloride solution is within the error of the technic for the determination of sodium by the method used. The difference between the magnesium contents of the unwashed corpuscles and of the magnesium washed corpuscles is only 2.5 per cent of the magnesium content of the wash solution. This could be accounted for by the supernatant wash solution that it was not possible to remove with the pipette. The small figure indicates not only that there was probably no

adsorption of magnesium at the surface of the corpuscles during the time that they were in the magnesium solution, which was approximately one hour, but also that the contact between corpuscles after this degree of centrifuging is a very close one, fitting Krogh's idea of the infinitesimal resistance which the corpuscle offers to contortion, and indicating that the continued decrease in volume which is obtained with increased centrifuging is due to squeezing the corpuscle rather than to packing.

The difference between the potassium contents of the control and of the potassium-washed corpuscles is greater than could be accounted for by the fluid present, and indicates that the potassium has either been adsorbed at the surface of the corpuscles, or has entered them to some extent during the process of washing. That the potassium has entered

TABLE 1

Metal content and volume changes of washed corpuscles

	VOLUME CC.	MGM. FOR EACH 100 CC. OF CORPUSCLES			
		Potassium	Sodium	Magnesium	Calcium
Unwashed	2.25	450	0	0	
Washed in potassium chloride solution	2.25	555			
Washed in magnesium chloride solution	2.25	470		8.9	
Washed in sodium chloride solution	2.20	420	0		
Unwashed	4.2	335			
Washed in sodium chloride solution	4.3	315			
Unwashed		291			
Washed in potassium chloride solution		386			
Washed in solution containing calcium					16
Washed in solution containing calcium					19
Washed in solution containing calcium					23
Washed in solution containing calcium					9

the corpuscle would seem to be the more probable alternative, for since we have evidence that potassium can leave the corpuscle fairly easily, it is safe to assume that it can also enter it. Meyer and Short, and also Wilkins and Kramer, call attention to the importance of not allowing serum to stand in contact with the clot for longer than two hours before separating it for the potassium determination, if an abnormally high serum potassium resulting from passage of potassium from the corpuscles is to be avoided. With the preliminary centrifuging for half an hour, the corpuscle potassium contents have on the whole been lower than those given by Kramer and Tisdall for blood corpuscles, and the potassium serum content has, in a few instances in which it has been tested, proved to be high; this is probably accounted for by an excessive loss of potassium during centrifuging. It is probable that on account of this initial loss

having already taken place, corpuscles washed with sodium chloride solution show only a slight further loss over the corpuscles centrifuged in their serum. The higher potassium content of the magnesium-washed corpuscles over those centrifuged in their serum may be due to an inhibiting effect of the bivalent metal.

The calcium found in the corpuscles after washing was also greater than would be accounted for by the amount of fluid left after removal of as much as possible with the capilliary pipette, which amount would be something less than 0.1 cc. for each 3 or 4 cc. of corpuscles. The initial calcium content of the corpuscles was not run in these instances, but in other instances in which I have tested for the calcium content of normal corpuscles by first removing the serum and then hemolyzing and treating with acid before precipitating with trichloracetic acid, I have obtained a calcium content over and above the blank on all materials used in the process, that would not seem to be accounted for by the trace of serum present, but which I believe appears as the result of a more complete destruction of the stroma before precipitation of the protein substances than is obtained by the Kramer and Tisdall method of hemolyzing the whole blood with 25 cc. of water. This amount has varied from 1 to 3 mgm. for each 100 cc. of corpuscles. The average amount of calcium found in the corpuscles after washing with a solution containing 83 mgm. calcium for each 100 cc. of water was 16.2 mgm. for each 100 cc. This comparatively large amount of calcium, nearly 20 per cent of the calcium in the wash solution, is either adsorbed at the surface of the corpuscles or enters them during the process of washing. The further change in the calcium content on more prolonged treatment would suggest the latter. Practically no change in volume occurred as a result of washing, although the corpuscles were exposed to the wash solutions for about one hour. This is not in agreement with the finding of Ege who noted a 10 per cent increase in volume in rabbit and sheep corpuscles exposed to isotonic sodium chloride solution for two hours, and subsequently centrifuged for fifteen minutes. This disagreement is probably due to the different type of corpuscle used and the shorter time of exposure, as I have noted this increase in size described by Ege, on longer exposure of human corpuscles at room temperature. On a forty-eight hour exposure of rabbit corpuscles to isotonic sodium chloride solution, Ege obtained the decrease in volume which I describe later for human blood corpuscles incubated at 36°C.

DATA OBTAINED BY INCUBATING CORPUSCLES IN SODIUM CHLORID FOR VARYING LENGTHS OF TIME. *Protocol 2.* Ten cubic centimeter portions of group 4 blood were mixed with 100 cc. portions of sterile isotonic sodium chloride solution at a pH of approximately 7.4 and incubated for one, two, three and four days respectively; an unwashed portion was used as

a control. Sodium and potassium determinations were made, and resistance tests were run. Hemolysis was not evident until the fourth day of incubation (table 2).

Protocol 3. Seven cubic centimeter portions of group 4 citrated blood were treated in a manner similar to that of protocol 2, except that in the process of incubation they were exposed to a temperature of 40°C. After

TABLE 2

Corpuscles incubated for different lengths of time in isotonic sodium chloride solution; volume, resistance and metal content changes

SERIAL NUMBER	DAYS INCUBATED	FINAL VOLUME CC.	MGM. FOR EACH 100 CC. CORPUSCLES		RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT																
			Potassium	Sodium	0.03	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48
(1)	0	4.3	326	0										4	3	2	1	1	1	0
(2)	1	4.1	247	0										4	3	2	2	1	1	1
(3)	2	4.2	188	63				4	3	3	3	3	3	3	2	2	2	1	1	1
(4)	3	3.6	3	151	4	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
(5)	4		0	188					4	3	3	2	2	2	1	1	1	1	1	1

TABLE 3

Corpuscles incubated for different lengths of time in sodium chloride solution; volume, resistance and metal content changes

SERIAL NUMBER	DAYS INCUBATED	FINAL VOLUME, CC.	MGM. FOR EACH 100 CC. CORPUSCLES		RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT															
			Potassium	Sodium	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48
(1)	0												4	3	2	2	1	1	0
(2)	2	3.1	396	49								4	3	3	2	2	1	1	Trace
(3)	3	2.4	33	160	4	3	2	1	1	1	1	1	1	1	1	1	1	1	1
(4)	4		129	127	4	3	2	2	1	1	1	1	1	1	1	1	1	1	1

four days' incubation, there was evident release of hemoglobin into the medium (table 3).

Protocol 4. Ten cubic centimeter portions of citrated blood were placed in 100 cc. portions of sterile isotonic sodium chloride solution. One was immediately condensed and centrifuged, one was placed in the ice box for one day, one was incubated for one day, and one for two days, and afterwards centrifuged for the standard time to obtain volume changes. There was no hemolysis (table 4).

DATA OBTAINED BY INCUBATING CORPUSCLES IN POTASSIUM CHLORIDE SOLUTION FOR VARYING LENGTHS OF TIME. *Protocol 5.* Four 10 cc. portions of citrated blood were added to 100 cc. portions of potassium chloride solution. One was immediately centrifuged, one placed in the ice box for one day, one in the incubator for the same length of time, and the fourth incubated for two days. A 10 cc. portion of blood was centrifuged immediately, without washing. No evident hemolysis occurred (table 5).

TABLE 4

Corpuscles incubated for different lengths of time in sodium chloride solution; volume, resistance and metal content changes

SERIAL NUMBER	DAYS INCUBATED	FINAL VOLUME, CC.	MGM. FOR EACH 100 CC. CORPUSCLES		RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT									
			Potassium	Sodium	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48	0.51
(1)	0	4.2	335	28			4	3	2	1	0				
(2)	0	4.3	315												
(3)	1 (icebox)	4.5	267	44			4	3	2	1	1	1	0		
(4)	1 (incubator)	4.7	252	72			4	3	2	1	1	1	1	1	0
(5)	2 (incubator)	5.1	243	115	4	3	3	2	1	1	1	1	1	1	1

TABLE 5

Corpuscles incubated for different lengths of time in isotonic potassium chloride solution; volume, resistance and metal content changes

SERIAL NUMBER	DAYS INCUBATED	FINAL VOLUME, CC.	MGM. FOR EACH 100 CC. CORPUSCLES	RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT							
			Potassium	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48
	Control	4.1	291	4	3	2	1	1	Trace	0	
(1)	1 (washed)	4.1	386								
(2)	1 (icebox)	4.2	430			3	2	1	Trace	Trace	0
(3)	1 (incubator)	4.8	470			4	3	2	1	1	Trace
(4)	2	5.5	462					4	3	2	2

THE RESULT OF INCUBATING CORPUSCLES IN VARIOUS MIXTURES OF ISOTONIC SODIUM AND POTASSIUM CHLORIDS FOR A UNIFORM LENGTH OF TIME. *Protocol 6.* Eight cubic centimeter portions of group 4 citrated blood were incubated for three days at 36°C. in 100 cc. portions of mixtures of isotonic sodium and potassium chlorides. One unwashed portion was used as a control. No hemolysis occurred in the course of incubation and centrifuging, except in the instance of the specimen incubated in the 2:8 mixture of isotonic sodium chloride and isotonic potassium chloride solution (table 6).

Protocol 7. Seven cubic centimeter portions of group 4 citrated blood were mixed with 100 cc. portions of mixtures of isotonic potassium and sodium chloride solutions, the porportions of sodium chlorid to potassium chlorid being 8:2, 6:4, 5:5, 4:6, 2:8. These were incubated for three days at 40°C. There was no hemolysis in the 8:2 mixtures. There was considerable hemolysis in the 2:8 mixture. In the remaining three prepara-

TABLE 6

Corpuscles incubated three days in mixtures of isotonic sodium and potassium chloride solutions; volume, resistance and metal content changed

SERIAL NUMBER	PARTS OF ISOTONIC		FINAL VOLUME, CC.	MGM. FOR EACH 100 CC. CORPUSCLES		RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT														
	Sodium chlorid	Potassium chlorid		Sodium	Potassium	0.03	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45
(1)	8	2	2.5	27	83	4	3	3	2	1	1	1	1	1	1	1	1	1	1	1
(2)	4	6	2.5	35	188			4	3	3	2	2	1	1	1	1	1	1	1	1
(3)	5	5	2.65	30	191					4	3	2	2	1	1	1	1	1	1	1
(4)	6	4	3.0	47	285					4	3	3	2	2	1	1	1	1	1	1
(5)	7	3	3.1	19	286								4	3	2	2	1	1	1	1
(6)	8	2		72	418										4	3	3	2	2	1
(7)	Initial		2.8		362									4	3	2	1	1	1	0

TABLE 7

Corpuscles incubated three days in mixtures of isotonic sodium and potassium chloride solutions; volume, resistance and metal content changes

SERIAL NUMBER	PARTS OF ISOTONIC		FINAL VOLUME, CC.	MGM. FOR EACH 100 CC. CORPUSCLES		RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT														
	Sodium chlorid	Potassium chlorid		Sodium	Potassium	0.03	0.06	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.39	0.42	0.45	0.48	
(1)	8	2	2.4	228	205	4	3	3	2	1	1	1	1	1	1	1	1	1	1	
(2)	6	4	2.3	101	276					4	3	2	1	1	1	1	1	1	1	
(3)	5	5	2.5	115	332						4	2	2	1	1	1	1	1	1	
(4)	4	6	2.8	37	415							4	3	2	1	1	1	1	1	
(5)	2	8		25	490										4	2	1	1	1	
(6)	Initial resistance													4	3	2	2	1	1	

tions there was a faint reddening which was estimated from the depth of the color and the amount of fluid present to be insufficient to affect the volume determination to the first decimal place. The same blood was used for protocols 3 and 7 (table 7).

THE RESULT OF INCUBATING BLOOD IN ISOTONIC MAGNESIUM CHLORIDE SOLUTION DILUTED TO VARIOUS DEGREES WITH ISOTONIC SODIUM CHLORIDE SOLUTION. *Protocol 8.* Five cubic centimeter portions of defibrinated blood were added to 100 cc. portions of isotonic magnesium chloride solution, and mixtures of isotonic magnesium and sodium chloride solutions

TABLE 8

Corpuscles incubated three days in mixtures of isotonic magnesium and sodium chloride solutions; volume, resistance and metal content changes

SERIAL NUMBER	PARTS OF ISOTONIC		FINAL VOLUME, CC.	MGM. FOR EACH 100 CC. CORPUSCLES			RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT														
	Magnesium chlorid	Sodium chlorid		Magnesium	Potassium	Sodium	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48
(1)	100	0	1.43	79	64	0		4	3	3	2	2	1	1	1	1	1	1	1	1	1
(2)	50	50	1.63	57	60	149				4	3	3	3	2	2	1	1	1	1	1	1
(3)	40	60	1.83	21	112	51	4	3	3	3	2	2	2	1	1	1	1	1	1	1	1
(4)	20	80	1.95	6	88	40		4	3	3	2	2	2	1	1	1	1	1	1	1	1
(5)	10	90	2.10	4	74	91			4	3	2	2	1	1	1	1	1	1	1	1	1
(6)	5	95		6	44	41			4	3	2	2	1	1	1	1	1	1	1	1	1
(7)	Initial		2.25	0	374	0									4	3	3	2	1	1	0
(8)	Washed		2.25	9																	

TABLE 9

Corpuscles incubated two days in mixtures of isotonic magnesium and sodium chloride; change in resistance and metal content

SERIAL NUMBER	PARTS OF ISOTONIC		MGM. FOR EACH 100 CC. CORPUSCLES		RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT														
	Magnesium chlorid	Sodium chlorid	Magnesium	Sodium	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45
(1)	100	0	108		3	3	2	2	2	2	2	2	2	1	1	1	1	1
(2)	50	50	64	121			4	3	3	3	3	3	3	2	2	1	1	1
(3)	20	80	21	145			4	3	3	3	3	2	2	2	1	1	1	1
(4)	10	90	9						4	3	3	3		2		1	1	1
(5)	5	95	7	291					4	3	3	3		2		1	1	1
	0	100	0	274					4	3	3	3		2		1	1	1
(6)	Initial resistance										4	3	3	2	2	1	1	0

in the proportions of 50:50, 40:60, 20:80, 10:90, 5:95. These were incubated for three days at 36°C. As controls, unwashed corpuscles and corpuscles washed with isotonic magnesium chloride solution were used. During the course of the work no hemolysis occurred except in the 10:90 magnesium to potassium mixture, in which there was a faint trace of red

in the supernatant fluid, which was calculated not to alter materially the volume of the remaining corpuscles, and in the 5:95 mixture in which the hemolysis was great enough to invalidate a change of volume determination (table 8).

Protocol 9. Five cubic centimeter portions of citrated group 1 blood were added to 100 cc. portions of isotonic magnesium chloride and sodium chloride solutions, and to mixtures of these solutions in the proportion of 50:50, 80:20, 90:10 and 95:5. These preparations were incubated for two days. During the course of incubation some of the specimens clotted, which entailed the loss of a certain amount of corpuscle substance in the clot, and prevented the determination of changes in volume (table 9).

THE RESULT OF INCUBATING BLOOD IN ISOTONIC MAGNESIUM CHLORIDE SOLUTION DILUTED TO VARIOUS DEGREES WITH ISOTONIC POTASSIUM CHLORIDE SOLUTION. *Protocol 10.* Five cubic centimeter portions of defib-

TABLE 10

Corpuscles incubated three days in mixtures of isotonic magnesium and potassium chlorids; change in volume, resistance and metal content

SERIAL NUMBER	PARTS OF ISOTONIC		FINAL VOLUME, CC.	MGM. FOR EACH 100 CC. CORPUSCLES		RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT														
	Magnesium chlorid	Potassium chlorid		Magnesium	Potassium	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48	0.51
(1)	100	0	1.43	79	64	4	3	3	2	2	1	1	1	1	1	1	1	1	1	1
(2)	50	50	1.97	48	437				4	3	3	3	2	2	1	1	1	1	1	1
(3)	40	60	2.03	47	528								4	3	3	2	2	1	1	1
(4)	10	90	2.70	12	536												4	3	3	2
(5)	5	95		6	605														4	3
(6)	Initial		2.25	0	375								4	3	3	2	1	1	1	0
(7)	Washed*		2.25	9																

* With isotonic magnesium chloride solution.

rinated blood from the same specimen used in the experiment described in protocol 8 were added to 100 cc. portions of isotonic magnesium chloride solution and mixtures of isotonic magnesium and potassium chloride solutions in the proportions of 50:50, 40:60, 10:90 and 5:95 These were incubated for three days at 36°C. An unwashed control and a control washed with isotonic magnesium chloride were prepared (table 10).

Protocol 11. Eight cubic centimeter portions of citrated blood were incubated in 100 cc. portions of isotonic magnesium chloride solution and mixtures of isotonic magnesium and potassium chloride solutions, mixed in the proportions of 50:50, 20:80, 9:91 and 4.7:95.3. These were incubated for two days. As some clotting took place, the change in volume could not be determined (table11).

THE RESULT OF INCUBATING BLOOD IN ISOTONIC CALCIUM CHLORIDE SOLUTION DILUTED TO VARIOUS DEGREES WITH ISOTONIC SODIUM CHLORIDE AND WITH ISOTONIC POTASSIUM CHLORIDE SOLUTIONS. *Protocol 12.* Cor-

TABLE 11

Corpuscles incubated two days in mixtures of isotonic magnesium and potassium chlorids; change in resistance in metal contents

SERIAL NUMBER	PARTS OF ISOTONIC		MGM. FOR EACH 100 CC. CORPUSCLES		RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT															
	Magnesium chlorid	Potassium chlorid	Magnesium	Potassium	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48
(1)	100	0	46	260	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1
(2)	50	50	21	270						4	3	3	2	2	1	1	1	1	1
(3)	20	80	13	420								4	3	3	2	2	1	1	1
(4)	9	91	6	440									4	3	3	3	2	1	1
(5)	4.7	95.3	3	500									4	3	3	2	1	1	1
(6)	Initial		5	360								4	3	2	1	1	1	1	0

TABLE 12

Change in calcium content of corpuscles incubated in medium containing calcium

	MGM. FOR EACH 100 CC.			RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT												
	Determined		Calculated													
	Corpuscles	Fluid and serum	Fluid	0.06	0.09	0.12	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42
Initial resistance										4	3	2	1	1	0
Washed	16														
Incubated	123	79.5	83	4	2	2	1	1	1	1	1	1	1	1	1
Initial resistance										4	3	1	1	1	0
Washed	19	74	83												
Incubated	122			3	2	2	2	2	1	1	1	1	1	1	1
Initial resistance											4	3	2	1	1
Washed	23.4		83												
Incubated	120			1	1	1	1	1	1	1	1	1	1	1	1
Incubated	123	80	83												
Washed	9		83												
Incubated	129														

puscles were washed with a 1:4 mixture of isotonic calcium and isotonic sodium chloride solutions. Corpuscles were incubated for two days in the same mixture, during which time much hemolysis took place. Cal-

cium determinations were made on the corpuscles, and the supernatant fluid after incubation. A comparison is made between these determinations and the number of milligrams for each 100 cc. calculated to be present in the fluid to which the blood was added. The discrepancies

TABLE 13

Comparison of calcium content of corpuscles after incubation in various mixtures of isotonic sodium and calcium chloride solutions with that of the media

PARTS OF ISOTONIC		CALCIUM, MGM. FOR EACH 100 CC.			POTASSIUM, MGM. FOR EACH 100 CC. CORPUSCLES	RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT												
		Determined		Calculated														
Calcium chlorid	Sodium chlorid	Corpuscles	Fluid and serum	Fluid		0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45
1	2	134	119	138	38	3	2	1	1	1	1	1	1	1	1	1	1	1	1
1	4	109		83	76	3	2	1	1	1	1	1	1	1	1	1	1	1	1
1	8	82	42	46	210		4	2	2	1	1	1	1	1	1	1	1	1	1
1	16	65	26	24	264		4	2	2	1	1	1	1	1	1	1	1	1	1
Average control.....													4	3	2	1	1	0	

TABLE 14

Comparison of calcium content after incubation in mixtures of isotonic calcium and potassium chlorids with that of the media

PARTS OF ISOTONIC		CALCIUM, MGM. FOR EACH 100 CC.			RESISTANCE TEST, SODIUM CHLORIDE SOLUTION, PER CENT									
		Determined		Calculated										
Calcium chlorid	Potassium chlorid	Corpuscles	Fluid and serum	Fluid	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48
1	4	78	70	82			4	3	2	2	2	1	1	1
1	7	43		52	4	3	3	2	2	2	2	1	1	1
1	10	26	36.6	37.2		4	3	3	2	2	2	1	1	1
Initial resistance								4	3	2	1	1	1	0
1	7	37	46	52					4	3	3	3	2	2
1	10	24		37.2	4	3	3	3	3	2	2	2	2	1
1	4	82		82							4	2	1	1

that occur between the calcium values found in the supernatant fluid and that determined by calculation are probably in part due to dilution with serum and débris from hemolyzed corpuscles. These five tests were made on different blood specimens at widely different times (table 12).

Protocol 13. Work similar to that described in protocol 12 was done on blood incubated for two days in mixtures of isotonic calcium and sodium chloride solutions made in the proportions of 1:2, 1:4, 1:8 and 1:16. The control resistance determination of the blood used was not recorded, so an average normal resistance is included below (table 13).

Protocol 14. Mixtures of isotonic calcium and potassium chloride solutions were made and blood was incubated in them for two days. Three different sets were run. In a mixture of potassium and calcium chloride, corpuscles deteriorated extensively, and there was a great deal of hemolysis (table 14).

ANALYSIS OF DATA. The foregoing data have been analyzed from three points: *1*, to see what relationship the metal content of the corpuscles bore to changes in resistance; *2*, to compare the amount of metal present in the corpuscles with that present in the medium, in order to see if under these drastic conditions there were any differences in the tendencies of the metals to enter the corpucles; and *3*, to see if there was any correlation between change in resistance of the corpuscles, change in volume, and their metal content.

The relative ability of potassium, sodium, calcium and magnesium to permeate the corpuscle. For the sake of convenience I have first considered the evidence regarding the relative tendencies for sodium, potassium, magnesium and calcium to enter the corpuscle. Series in which the incubation times have been uniform, but in which the amount of metal present in the incubating fluid has been varied, have been made use of in this connection. In order to check the possible effect of the presence of the second metal used as a diluent on the permeability of the one under consideration, two diluents have been used. As the corpuscles changed appreciably in volume during incubation, their metal content has been calculated both on the basis of their original volume and of their final volume. As a basis of comparison of the four metals, the metal contents of the corpuscles and of the medium have been plotted as molar equivalents. These are given in figures 1, 2 and 3.

In figure 1 the potassium contents of corpuscles that have been incubated in an isotonic solution of potassium chloride, diluted with sodium chlorid (curves *6* and *7*) and with magnesium chloride (curves *10* and *11*), are compared with the potassium content of the fluids in which the corpuscles were incubated. These curves have been derived from data given in protocols 6, 7, 10 and 11. It will be seen that there is a general tendency for the amount of potassium in the corpuscles to equal that in the medium. The initial potassium content of the corpuscles has been indicated on the chart in three instances, and it is evident that the corpuscles tended to lose potassium when it was present in the medium in amounts less than those present in the corpuscles, and to gain when it was present in the medium

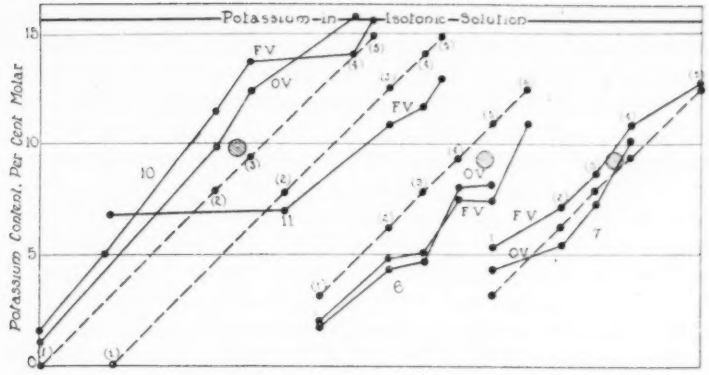


Fig. 1. Relation of the potassium content of the corpuscles to that of the medium *6 and 7:* data from protocols 6 and 7, corpuscles incubated in potassium chloride solutions diluted with sodium chloride solution. *10 and 11:* data from protocols 10 and 11, corpuscles incubated in potassium chloride solutions diluted with magnesium chloride solution.

——— Potassium in corpuscles. *O. V.*, calculation based on the original volume of the corpuscles. *F. V.*, calculations based on final volume of the corpuscles. ● initial potassium in corpuscles. ---------- Potassium in medium.

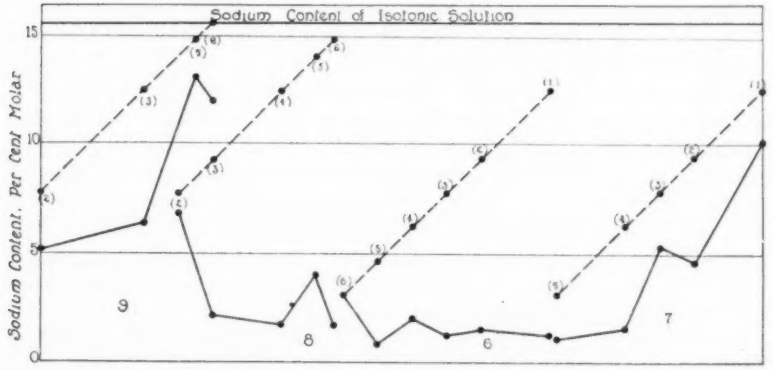


Fig. 2. Relation of the sodium content of the corpuscles to that of the medium *6 and 7:* data from protocols 6 and 7, corpuscles incubated in sodium chloride solutions diluted with potassium chloride solution. *8 and 9:* data from protocols 8 and 9, corpuscles incubated in sodium chloride solutions diluted with magnesium chloride solution.

——— Sodium in corpuscles. ---------- Sodium in medium.

in greater amounts This is true of the series incubated for three days, but although the members of the series incubated for two days in mixtures of potassium and magnesium showed an increase in their potassium content in an excess of potassium, they did not apparently lose so readily in the absence of potassium and the presence of magnesium. The tendency

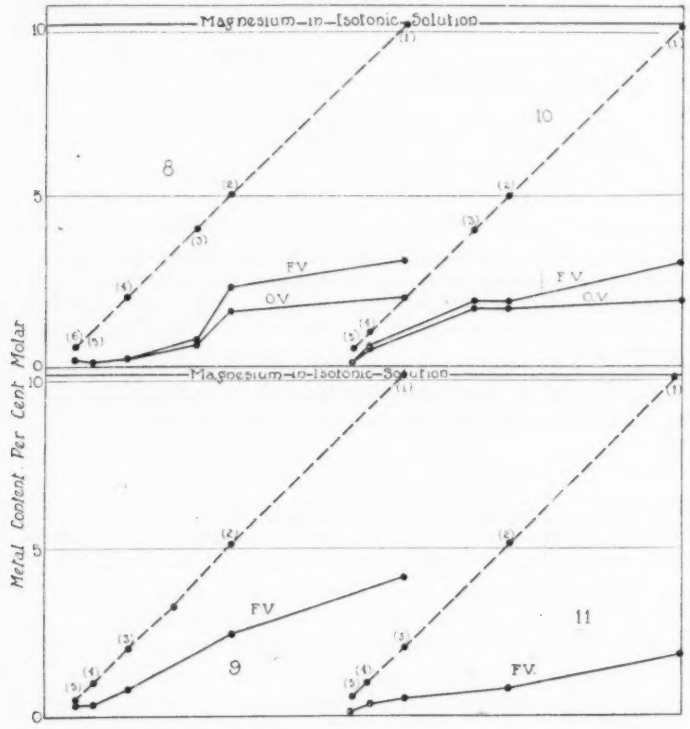


Fig. 3. Relation of the magnesium content of the corpuscles to that of the medium. *8 and 9:* data from protocols 8 and 9, corpuscles incubated in magnesium chloride solutions diluted with chloride solution.
———— Magnesium in corpuscles. *O. V.*, calculations based on original volume of corpuscles. *F. V.*, calculations based on final volume of corpuscles. ---------- Magnesium in medium.

shown by some series to have a potassium content of the corpuscles less than the content of the medium, and by other series one somewhat greater than the content of the medium, may be due to some intrinsic differences in the blood specimens, such as differences in the amount present of a constituent which is able to take up potassium, or it may be due to a

technical error. As compared with the results obtained from other metals, however, there is a definite tendency for the potassium to be found within the corpuscle in the same proportion in which it is present without.

The result of sodium determinations with respect to this point are summarized in figure 2. Curves *9* and *10* show a distinct tendency of the sodium content of the corpuscles to approach that of the medium. Curve *7*, however, is from a series prepared immediately before the series charted in curve *9*, which is suspected of having been subjected to 40°C. The series given in curves *6* and *8* were incubated at lower temperatures; in these there is no relationship betwen the sodium content of the corpuscles and the sodium content of the medium. The salt used as a diluent in curve *6* was potassium chloride; in curve *8* it was magnesium. These data would seem to indicate that the corpuscle membrane is not permeable to sodium except after considerable injury, whereas to potassium under the conditions of these experiments it is freely permeable. That this difference in permeability of the cell membrane to potassium and sodium respectively, is not due primarily to that greater ability of potassium to injure the cell membrane, which is claimed for it by Hoeber, but to an intrinsic ability of potassium to enter the cell more readily under these circumstances, is evident, as the identical blood specimens (curve *6* of fig. 1) which show a loss of potassium when incubated in the mediums in which the potassium content was decreased by a dilution with a sodium salt, do not show a corresponding gain in the sodium (curve *6* of fig. 2). The magnesium content of the corpuscle, which in figure 3 is compared with that originally present in the medium, is found to increase with an increase in the magnesium content of the medium, but is always very much less than that of the medium. Curves *8* and *10* (protocols 8 and 10) are comparable, as they are derived from data obtained as the result of three days' incubation of the same blood specimen. Curve *11* (protocol 11), which is derived from the changes in blood incubated in magnesium and potassium mixtures for two days, shows lower values than curve *10*. Curve *9* is also derived from material incubated only two days, but there is reason to suspect that the incubator had been at 40°C. which might account for the much higher magnesium values obtained.

Calcium as compared with magnesium enters the corpuscle freely. In protocol 12 the calcium contents of corpuscle samples washed with a 1:4 mixture of isotonic sodium and calcium chloride solutions are compared with the calcium content of similar samples incubated for two days in the same mixtures. These are also compared with the calcium found in the fluid after addition of the blood, and with the theoretic calcium value for the 1:4 mixture. The average calcium found in four series of washed corpuscles was 16.2 mgm. for each 100 cc. of corpuscles. The process of washing and centrifuging down in the solution takes longer than an hour.

The average calcium content in the incubated corpuscles of five series was 125.4 mgm. for each 160 cc. The average of three calcium content determinations of the fluids after mixing with blood was 77.6. The theoretic value of the calcium content in the solution with which the blood was mixed in each case was 83 mgm. for each 100 cc. There is a very great increase in the calcium content after incubation over that found after washing, which would point to the hypothesis that the calcium enters the corpuscles rather than that it is absorbed at their surfaces. The average incidence of calcium in the incubated corpuscles was 38 per cent greater than its incidence in the fluid with which they were surrounded. In the series given in protocol 13, through a wide variation in the amount of calcium in the fluid in which the corpuscles were incubated there is an excess of calcium found in the corpuscles over that found in the fluid, and this excess was greater in the lower dilutions of calcium, amounting, in the 1:8 dilutions for isotonic calcium and sodium chloride solution mixtures, approximately to 200 per cent. It would seem that not only is calcium able to enter the corpuscles, but that in some way, either physical or chemical, it is combined with some constituent of the corpuscles.

After incubating in mixtures of isotonic solutions of calcium and potassium chloride (protocol 14), corpuscles were found to have taken up, in proportion to their volume, amounts of calcium about equal to, or somewhat less than that in the medium, and markedly less than that taken up in mixtures of calcium and sodium salts. This might be accounted for in part by a difference in the size of the corpuscles in the two mediums, and a consequent difference in the concentration of the constituent that has an affinity for calcium, but could not be wholly accounted for in this way; it is more probable that it is due to the much greater deterioration of the corpuscles in the medium containing potassium.

The relationship between the metal contents of the corpuscle and the change in resistance. In figure 4 the results of incubating corpuscles in either isotonic sodium or potassium chloride solutions for different lengths of time, and in mixtures of these solutions for uniform lengths of time, are given. The sodium content of the corpuscles, the potassium content, the combined sodium and potassium contents, and the change in resistance, are plotted. As slight initial changes in resistance which are not necessarily in the direction of the final change occur as a result of washing the corpuscles, the resistances of the unwashed corpuscles have not been included in the charts. Slight changes in resistance from washing have already been reported by Brinkman, who considers them due to a change in the balance between the lecithin and cholesterol content. If this is so, it would appear that the inclusion of the resistance of the unwashed cell would introduce a factor which is foreign to the question under consideration. Since after incubation there is some hemolysis in the lower

dilutions, in order graphically to portray the change, the highest dilution (giving 1 in a scale in which hemolysis is measured from 0 to 4) has been plotted as the upper limit of the range as given in the chart, and the highest dilution giving any gross evidence of cells, namely 3, has been plotted as the lower limit. The metal contents are given as per cent molar values, based on the final mass of the corpuscles tested.

On comparing the potassium and sodium contents of the corpuscles with the degree of resistance, it appears that there is a definite tendency for the

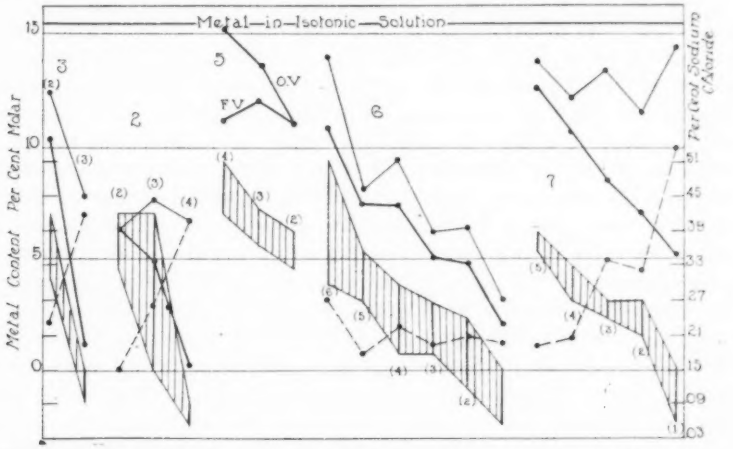


Fig. 4. Relation of change in resistance of corpuscles to change in their potassium sodium, and total metal content. *2 and 3:* data from protocols 2 and 3, corpuscles incubated for different lengths of time in isotonic sodium chloride solution. *5.* Data from protocol 5, corpuscles incubated for different lengths of time in isotonic potassium chloride solution. *6 and 7:* data from protocols 6 and 7, corpuscles incubated for the same length of time in various mixtures of isotonic sodium and potassium chloride solutions.

——— Potassium content of corpuscles. ——— Sodium content. ——— Total metal. Hatched area indicates range of resistance of corpuscles as indicated by the lowest per cent of sodium chloride in which "1" and "3" appear.

potassium content to parallel the degree of fragility, while the sodium content bears no relationship to it, and may be either high or low, with a greatly increased resistance. The combined molar values of the two metals, which, assuming the metals to be cations, would be an index of their osmotic pressure, or according to Loeb's work, since these metals are both univalent, of the swelling capacity of the corpuscular proteinates, also do not show any relationship to changes in resistance. In the consideration of the effect of potassium and sodium respectively on fragility, there is found a parallelism between fragility and potassium content only.

Sodium is either entirely passive, or else protects against the increase in fragility caused by potassium. In the preparations summarized in protocol 7, in which high sodium content was found in the corpuscles, there occurred in one instance 205 mgm. of potassium for each 100 cc. of corpuscles, with a resistance to hypotonic salt solution which was equal to that of corpuscles described in protocol 6, which had a potassium content of only 83 mgm. for each 100 cc. This may or may not be due to individualities of the corpuscles, which are independent of the metal contents.

With respect to the bivalent metals, magnesium and calcium, the situation seems to be different. It is difficult to express this point graphically, but examination of the protocols will show that when the magnesium content of the corpuscle is high, the potassium content may be high with a considerable degree of resistance. If protocol 10, for instance, is examined for this point, it will be seen that the corpuscles incubated in the 50:50 mixtures of isotonic potassium and calcium chlorides contained a definite excess of potassium over that of the unwashed control corpuscles, and at the same time gave a marked increase in resistance. The magnesium content in these corpuscles is 62 mgm. for each 100 cc. In protocol 9, in the presence of 306 mgm. of potassium with 108 mgm. of magnesium, there is marked increase in resistance over that of the blood specimen, in which there is only 295 mgm. of potassium with 7 mgm. of magnesium. Although potassium determinations were not made in the case of the calcium treated corpuscles except in one series in which there was a comparatively high potassium content with an increased resistance, as an increase in resistance took place in some instances in which the potassium content of the fluid in which the corpuscles were incubated was higher than that of normal corpuscles, it would seem evident that the effect of calcium on resistance is opposite to that of potassium. It would seem that both magnesium and calcium actively cause an increase in resistance, and the reason that this is not so evident in the case of magnesium, and was not brought out by the work reported in the second study of this series, is probably owing to the fact that magnesium enters the cell less readily under the circumstances of these experiments than does calcium.

Correlation of change in volume of the corpuscles with their metal contents. A study in change of volume by the hematocrit method is admittedly inaccurate, particularly when, as was the case in some of the preceding studies, it is not possible to centrifuge the blood specimens simultaneously, and an absolute regulation of the speed of the centrifuge is impracticable. The changes in volume which have occurred, however, are great and would seem to be significant. As it was found that no change in volume attributable to osmotic pressure differences resulted from washing with the salt solutions used in treating the corpuscles, data on volume and metal content obtained from the untreated corpuscles have been included in these

comparisons. I suspect, however, that there is a certain amount of exchange of comparatively uncombined potassium, which does not affect the physical state of the corpuscle and on that account the inclusion of data from the untreated corpuscles in the comparison might not be perfectly just. As the effect of the metal content on the change in volume is the point of interest, the metal contents have been plotted in terms of molar percentages, based on the original volumes of the corpuscle masses. The change in volume has been indicated as a percentage of the original

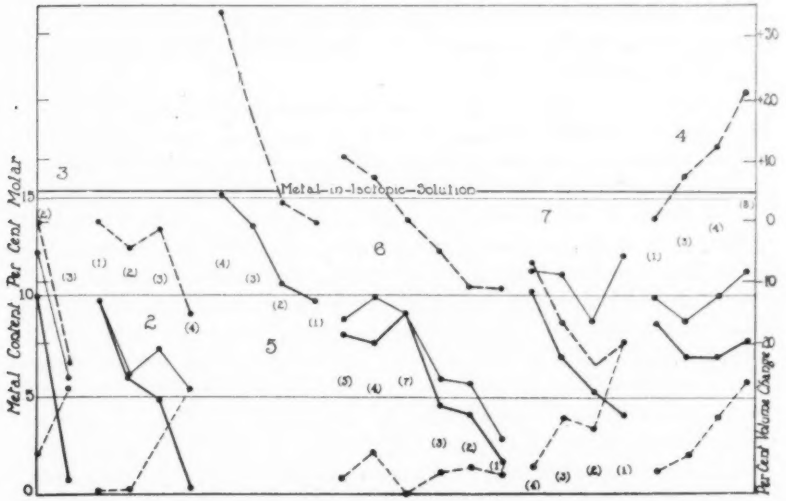


Fig. 5. Relation of change in volume of corpuscles to their change in metal content. *2, 3 and 4:* data from protocols 2, 3 and 4, corpuscles incubated for different lengths of time in isotonic sodium chloride solution. *5:* data from protocol 5, corpuscles incubated for different lengths of time in isotonic potassium chloride solution. *6 and 7:* data from protocols 6 and 7, corpuscles incubated for the same length of time in various mixtures of sodium and potassium chloride solutions. – – – Per cent change in volume. ——— Potassium content of corpuscles. ---------- Sodium content of corpuscles. ——— Total metal content.

volume, except in curves *3* and *7* of figure 5, when no initial blood volumes were obtained, but for which the volume probably nearest to the original volume was used as a basis of comparison.

If curves *6* and *7* of figure 5, the data for which, with the exception of (*7*) in curve *6*, were obtained from corpuscles incubated in mixtures of sodium and potassium chloride solutions for three days, are examined, it will be seen that there is a general tendency for the volume to parallel the potassium content. This would be even more marked if the reading (*7*) which

is from the unincubated control specimen had been omitted. In *6*, however, the sodium content is so low that the combined values of the sodium and potassium contents are practically equal to the potassium content, so that here we may merely have the expression of the effect of a univalent metal as such. In *7*, the volume change parallels the potassium content until the combined potassium and sodium content is great (*1*), when, if the result is not due to errors in volume determination, the combined content of sodium and potassium somewhat affects the corpuscle volume although merely as univalent cations their effect cannot be great, since at this reduced volume the combined metal content is greater than it is at the maximal volume of the corpuscles (*4*). The effect of the potassium would seem to predominate greatly.

In curves *2*, *3*, *4* and *5* (fig. 5), are represented results from corpuscles incubated for different lengths of time in the same salt solutions. In curve *5*, obtained from blood incubated in potassium chloride solution, the corpuscle volume closely follows the potassium content. The same is true of curve *3*, which represents the data from blood in which little sodium entered the corpuscles, and for which the combined values and the potassium values are approximately equal. In curve *2* at point (*3*), the volume would seem to be influenced more by the combined value of the univalent metals than by the potassium content; otherwise, here also the change-in-volume curve parallels the potassium-content curve. Curves *7* would seem to offer an exception. These are derived from the control blood specimen (*1*), blood kept in the ice box for one day (*2*), and two incubated specimens, (*4*) and (*5*). In these curves no relationship between corpuscle volume change and potassium content is seen. Judging from a small amount of other data which I have on this point, it would seem probable that a different order of change takes place in the corpuscles at temperatures widely differing from body temperature which, if so, would make it inadvisable to include (*2*) in these data.

It would appear that there is a definite tendency for change in corpuscle volume to run parallel with change in potassium content of the corpuscle, and this would seem to be not wholly due to the fact that potassium was usually in the corpuscle in a preponderance. These data would seem to indicate that although there may be some effect due to univalent cations, as such, on the stroma hemoglobin complex, potassium may have an independent and different ability to determine the volume of human corpuscles.

Of the bivalent metals magnesium only was studied quantitatively with respect to volume change, as calcium, strontium and barium all caused much hemolysis to human corpuscles incubated in solutions of their salts. The comparisons between the volume changes and the metal contents are made in figure 6, derived from protocols 8 and 10. With the exception of

(7), data on the magnesium-washed control specimen, these data are derived from corpuscles incubated for three days in isotonic magnesium chloride solution, and in solutions in which the magnesium content was reduced by dilution with isotonic sodium chloride solution (curves *8*) and with isotonic potassium chloride solution (curves *10*). In order graphically to portray magnesium in its rôle of a molecule, and also in its rôle as a bivalent metal, the magnesium content has been plotted as a

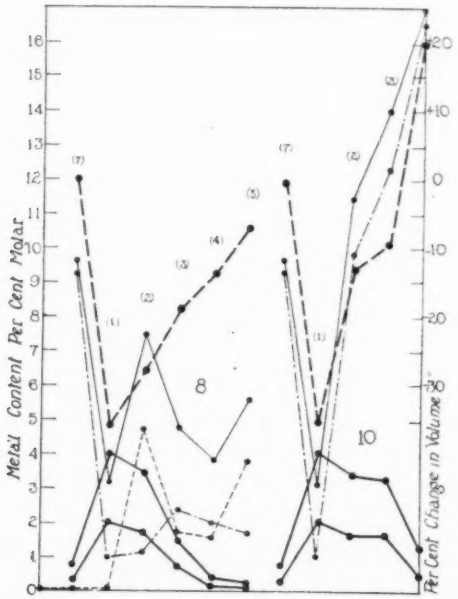


Fig. 6. Relation of change in volume of corpuscles to the change in their magnesium content. *8:* data from protocol 8, corpuscles incubated in magnesium chloride solutions diluted with sodium chloride solution. *9:* data from protocol 9, corpuscles incubated in magnesium chloride solutions diluted with potassium chloride solution. ——— Per cent change in volume. ——— Magnesium content of corpuscles. —·—·—·— Potassium content. ------- Sodium content. ——— Total metal content of corpuscles.

molar equivalent and above this curve has been drawn the curve of magnesium content in terms of its combining value. Potassium and sodium have been plotted as molar equivalents, and the combined molar values of magnesium, potassium and sodium (curves *8*) and of magnesium and sodium (curves *10*) have been plotted. In both comparisons an inverse relationship is apparent between the change in volume and the magnesium content. In curves *10*, the potassium content bears an inverse relationship

to the magnesium content, and, as would be expected from the previous data on corpuscle volume with reference to potassium, the curve of potassium content parallels the curve of change in volume. In curves *8*, however, in which the potassium content is low, the change-of-volume curve still bears a decided inverse relationship to magnesium content, and does not seem to be affected either by the sodium content which is fairly high in places, or by the combined sodium and potassium content.

DISCUSSION AND CONCLUSIONS

Of the univalent metals, sodium and potassium, a marked parallelism has been found between the potassium content of the corpuscles and their resistance to hypotonic salt solution, the resistance being inversely proportional to the potassium content; while sodium, on the other hand, would seem to be passive with respect to causing changes in resistance. The corpuscle content of this latter element may be either high or low with an increased resistance to hypotonic salt solution. Similarly, a marked change in volume accompanied changes in the potassium content produced at approximately body temperatures, a greater volume accompanying a greater potassium content, while the correlated changes in the sodium content would imply either that sodium is entirely passive, or that its ability to cause increase in volume is very much less.

The human blood corpuscle, even under the drastic test of incubation for three days in sodium chloride solution, has been found to be very impermeable to sodium. It would seem probable that sodium is not found normally within the human corpuscle because of the impermeability of the corpuscle membrane to this element. On the other hand, there is evidence that potassium can enter and leave the corpuscle comparatively easily, and it would seem that potassium which is found in more than ten times as great a concentration within the corpuscle as in the serum is not so distributed because of the impermeability of the corpuscle membrane to that metal.

If, as seems probable, the changes in potassium content which accompany these changes in resistance and volume are responsible for them, the reaction of sodium and potassium respectively on the corpuscle substance must be a radically different one. This radical difference in the behavior of the two univalent metals is not such as would be expected to result from the formation of metal proteinates as judged from the work that has been done on the effect of these metals on osmotic pressure, swelling capacity, and so forth, of gelatine (9) and casein. It would seem probable that potassium is able to combine with the substance of the human corpuscle in a manner different from the combining ability of sodium. Such an ability to combine with the corpuscle substance would explain its presence in greater quantities in the corpuscle than in the

serum, without the necessity of postulating a cell membrane which is impermeable to it. Such an ability to combine would also explain the greater ability of potassium than sodium to pass through the cell membrane, assuming the modern conception that the cell membrane is intrinsically merely a part of the cell substance in which certain concentrations have taken place. This conception of a high potassium content of the corpuscle combined with a permeability of its membrane is in harmony with work reported by Meigs for smooth muscle. He found that with a high potassium content as compared with the plasma bathing its cells, smooth muscle did not behave with reference to changes in osmotic pressure as though a semipermeable membrane were functioning. That proteins are able to maintain a radical difference in metal content from that of their surrounding medium without the assistance of a differentially permeable membrane, has recently been shown by Loeb, Atchley and Palmer who dialyzed the serum of patients against their acetic fluid and found that the relative potassium contents remained unchanged. Since the distribution of potassium, as these authors give it, was not in proportion to their findings for protein content of the respective fluids, it must have been influenced by a qualitative factor, and in that respect is suggestive of the distribution of potassium between corpuscles and serum.

The greater ease in the reversibility of changes in resistance by substituting one solution for the other, which I reported as occurring during the course of incubation of corpuscles either in potassium or sodium chloride solutions, might be explained on the basis of a change in the substance of the corpuscle such that the reaction resulting in its union with potassium was more easily reversible, rather than by a change in the permeability of the corpuscle membrane to potassium.

With respect to the bivalent metals, magnesium and calcium, both show an ability to enter the corpuscle under the conditions of this experiment, but the ability of the former to enter the corpuscles is less than that of the latter. In the mixtures of isotonic calcium and sodium chlorides of a less toxic nature, as evidenced by less hemolysis during incubation, the corpuscle calcium content was found to be much greater than the calcium content of the fluid in which the corpuscles were incubated. It would seem possible that the low calcium content of the normal corpuscle is not due to an inability of the calcium to enter the corpuscle, but to its greater tendency to remain with the serum proteins.

The changes in resistance which occur when corpuscles are incubated in solutions containing magnesium or calcium are not entirely accounted for by loss of potassium, and would seem to be due to the effect of the magnesium or calcium. The same is probably true of a decrease in volume, which has been found to take place in corpuscles incubated in magnesium.

In this work a parallelism has been shown between change in volume of the corpuscle and change in its resistance to hypotonic salt solution, the more condensed corpuscles being the more resistant. Work, however, which is in progress, seems to indicate that this relationship may not necessarily hold.

On the assumption that the metals are present as cations, it would appear that the resistance of human blood corpuscles to hypotonic salt solution bears no relationship to the capacity of their metal content to exert osmotic pressure.

BIBLIOGRAPHY

(1) Ashby: This Journal, 1924, lxviii, 250.
(2) Brinkman: Physiol. Abstr., 1922–1923, vii, 419.
(3) Denis: Journ. Biol. Chem., 1920, xli, 363.
(4) Doisy and Eaton: Journ. Biol. Chem., 1921, xlvii, 377.
(5) Ege: Biochem. Zeitschr., 1921, cxv, 109.
(6) Grüber: Quoted by Hamburger and Bubanovic.
(7) Hamburger and Bubanovic: Arch. internat. de physiol., 1910–1911, x, 1.
(8) Kramer and Tisdall: Journ. Biol. Chem., 1922, liii, 241.
(9) Kramer and Tisdall: Journ. Biol. Chem., 1921, xlviii, 223.
(10) Krogh: The anatomy and physiology of capillaries. New Haven, 1922.
(11) Loeb: Protein and the theory of colloidal behavior. New York, 1922.
(12) Loeb, Atchley and Palmer: Journ. Gen. Physiol., 1921–1922, iv, 591.
(13) Meigs: This Journal, 1910–1911, xxvii, p. xvii.
(14) Meigs and Ryan: Journ. Biol. Chem., 1912, xi, 401.
(15) Meyers and Short: Journ. Biol. Chem., 1921, xlviii, 83.
(16) Pick: Klin. Wochenschr., 1922, i, 2188.
(17) Ritter: Personal communication.
(18) Tisdall: Journ. Biol. Chem., 1922, l, 329.
(19) Van Slyke, Wu and McLean: Journ. Biol. Chem., 1923, lvi, 765.
(20) Wilkins and Kramer: Arch. Int. Med., 1923, xxxi, 916.

A STUDY OF THE MECHANISM OF CHANGE IN RESISTANCE OF ERYTHROCYTES TO HYPOTONIC SALT SOLUTION

IV. On a Radical Difference in the Effect of Univalent Cations with Reference to Resistance of the Corpuscles of Certain Animals, which is Associated with Differences in the Normal Metal Content of the Respective Corpuscles; and the Similarity of the Effect of the Bivalent Cations

WINIFRED ASHBY

From the Division of Medicine, Mayo Clinic, Rochester, Minnesota

Received for publication March 10, 1924

As a result of a previous study (2) of the effect of cations on the resistance of human erythrocytes to hemolysis by hypotonic salt solution it was postulated, since changes in resistance occur with changes in potassium but not in sodium content, and since rubidium, which is chemically similar to potassium can replace it with the same effect, that potassium produces these changes in resistance through an ability to combine with the corpuscle substance in some manner not shared by sodium. It was further postulated that the human corpuscle has a higher potassium content than the serum because of this combining ability, as indicated by this peculiar relationship to the corpuscle substance, and not because of an impermeability of the corpuscle membrane to potassium. If such an hypothesis be true, the corpuscles of other animals having a natural, high potassium content should show the same relationship to the univalent metals with respect to changes in resistance as the human corpuscle, and the corpuscles of those species having a natural high sodium content should show a reversed relationship. To test this point a study was made of the effect of the univalent cations, potassium, rubidium, sodium and lithium, previously used in studying the changes in resistance of human corpuscles, on the corpuscles of the horse, the guinea pig and the rabbit, whose corpuscles, like those of man, have a higher potassium content than their serum. These results were compared with their effect on the corpuscles of the dog, the cat and the steer, whose corpuscles have a high sodium content and a low potassium content. As it was suspected from the previous study, that the alkaline earth metals produced an active increase in resistance of a different order from the changes produced in

the presence of the univalent metals, which was in some way associated with qualities inherent to either the alkaline earth group as such, or to bivalent metals in general, and not to a peculiar relationship between these metals and the substance of the human erythrocyte, the effect of incubation on the corpuscles from these various species in magnesium and calcium chlorid was also tested.

TECHNIC. Blood was taken from the animals, with or without ether anesthesia, depending on the kind of animal used. It was found, however, that ether was deleterious to the corpuscles and had to be used sparingly. The blood was, in most instances, defibrinated by slight shaking with glass beads. When citrate was used as an anticoagulent, it was found important not to use it in excess. Sterile technic was essential. As in the previous study, 1 to 10 mixtures of blood and the isotonic solution of the salt of the metal adjusted to a pH of approximately 7.4 were made. These were incubated for various lengths of time, depending on the kind of blood used, the object being to obtain the maximal effect from incubation in the medium under consideration, without causing a deterioration of the corpuscles which would mask this effect.

RESULTS. As has been pointed out (1), the amount of hemolysis which occurs during incubation and the degree of resistance attained by the surviving corpuscles, bear no relationship to one another. Hemolysis, as determined by the supernatant fluid, was noted in these experiments. This was of interest in view of Hoeber's work on the effect of cations on hemolysis, in which, by observing the depth of coloration of the supernatant fluid over corpuscles incubated in somewhat hypotonic solutions of chlorids of the univalent metals, he determined the following order: $Li<Na<Cs<Rb<K$. With respect to this point it was found that all corpuscles show the toxic effect of sodium, deterioration resulting in lysis and in an irreversible fragility, less rapidly than they show the toxic effect of potassium. In relation to toxicity, I found that sodium and potassium occupy the relative positions assigned them by Hoeber. But, unlike Hoeber, I found that rubidium produced hemolysis more rapidly than potassium, and that lithium was more toxic than sodium. Of the two bivalent metals, magnesium and calcium, magnesium generally showed a degree of toxicity, as determined by hemolysis into the supernatant fluid which was of approximately the same degree as that of sodium. Calcium, on the other hand, showed much variation with respect to the production of deterioration resulting in hemolysis. It was very active against the corpuscles of man, guinea pig and rabbit, although it did not produce hemolysis in the corpuscles of the steer and the horse in two days, which was the maximal time that the corpuscles of any of the species were exposed to it.

With respect to the change of resistance to hypotonic salt solution caused by incubation in solutions of the chlorids of the univalent metals, sodium, lithium, potassium and rubidium, it will be seen from table 1 that the corpuscles of the horse, the rabbit and the guinea pig behave with respect to these metals in exactly the same manner as do the corpuscles of man. They increase in fragility in potassium and in rubidium chlorids, and increase in resistance in sodium and lithium chlorids. The potassium content of the rabbit and horse corpuscles are given by Hoeber as 523 and 413 mgm., respectively, for each 100 cc. of corpuscles, the sodium content being negligible. The potassium content of guinea pig corpuscles, as determined by others, is equally high. My own determination on one specimen was 420 mgm. for each 100 cc. These figures are, of course, of the same order as those for human blood corpuscles. In these species in which the corpuscles have a potassium content of the same order as that of human blood corpuscles, the changes in fragility with respect to sodium, potassium, lithium and rubidium are found to be the same (table 1).

The changes in resistance of the corpuscles of the dog, the cat and the steer are diametrically opposite to those seen in the corpuscles of man (table 2). The resistance of the corpuscles of these animals increases in potassium and rubidium, and decreases in sodium and lithium. The sodium content of the corpuscles of the dog, the cat and the steer, as given by Hoeber is in round numbers 284, 270 and 226 mgm. for each 100 cc., respectively, the potassium content being 27, 25 and 72 mgm. for each 100 cc. A natural high sodium corpuscle content would seem to be associated with an increase in fragility on incubation in the presence of available sodium, and an increase in resistance in conditions under which sodium can be lost, as for instance during incubation in potassium and rubidium chlorid solutions. This is a situation exactly opposite to that found in the corpuscles of animals of the first series, in which, in man at least, it was shown that the absorption of rubidium or potassium caused an increase in fragility, and that a loss of potassium on incubation in sodium or lithium solutions caused an increase in resistance (table 2).

That the increase in fragility is not caused by some destructive effect of the sodium, but that it is due to a reversible change in the physical properties of the corpuscle, was determined in several instances by establishing the increased fragility produced by sodium chloride solution, and then transferring the corpuscles, so reduced in resistance, to potassium chloride solution, in which they more than regained their former resistance. In fact, the marked increase in resistance in the cat corpuscles in potassium chloride solution (table 2) was obtained in this way. Cat corpuscles, to which potassium is so toxic that, unless the incubation is performed at room temperature, the increase in resistance resulting

TABLE 1

Comparison of the effect of univalent metals, sodium, lithium, potassium and rubidium, on the resistance of the corpuscles of the horse, the rabbit and the guinea pig with their effect on the resistance of the corpuscles of man

CORPUSCLES INCUBATED IN	READING OF RESISTANCE TEST, SODIUM CHLORID, PER CENT																
	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48	0.51	0.54	0.57	0.60
Man:																		
Untreated								4	3	2	1	1	0					
Potassium chlorid for 3 days																	4	2
Rubidium chlorid for 3 days																	4	3
Lithium chlorid for 3 days						4	3	2	1	1	1	1	1	1	1	1	1	1
Sodium chloride for 3 days			4	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1
Horse:																		
Untreated											4	3	3	3	2	1	1	0
Potassium chlorid for 2 days																4	1	1
Rubidium chlorid for 2 days																4	2	1
Lithium chlorid for 2 days	4	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1	0	
Sodium chlorid for 2 days			4	3	2	2	1	1	1	1	1	1	1	1	1	1	0	
Rabbit:																		
Untreated											4	3	2	1	1	1	1	
Potassium chlorid for 2 days														4	3	3	1	
Rubidium chlorid for 2 days															4	3	2	
Lithium chlorid for 2 days								4	3	2	1	1	1	1	1	1	1	
Sodium chlorid for 2 days		4	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	
Guinea pig:																		
Untreated									4	3	2	1	1	0				
Potassium chlorid for 1 day												4	2	1	1	1	1	
Rubidium chlorid for 1 day													4	2	1	1	1	
Lithium chlorid for 1 day		4	3	3	3	3	3	2	2	1	1	1	1	1	1	1	1	
Sodium chlorid for 1 day	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	

TABLE 2

Comparison of the effect of sodium, lithium and rubidium on the resistance of the corpuscles of the dog, the cat and the steer, with their effect on the resistance of the corpuscles of man

CORPUSCLES INCUBATED IN	READING OF RESISTANCE TEST, SODIUM CHLORID, PER CENT																		
	0.03	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48	0.51	0.54	0.57	0.60
Man:																				
Untreated										4	3	2	1	1	0					
Potassium chlorid for 3 days																			4	2
Rubidium chlorid for 3 days																			4	3
Lithium chlorid for 3 days								4	3	2	1	1	1	1	1	1	1	1	1	1
Sodium chlorid for 3 days					4	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1
Dog:																				
Untreated										4	3	1	1	1	1	0				
Potassium chlorid for 1 day				4	2	2	1	1	1	1	1	1	1	1	1	1				
Rubidium chlorid for 1 day					4	3	2	1	1	1	1	1	1	1	1	1				
Lithium chlorid for 1 day													4	3	2	2				
Sodium chlorid for 1 day													4	3	2	1				
Cat:																				
Untreated														4	2	1	0			
Potassium chlorid	4	3	3	2	2	2	2	2	1	1	1	1	1	1	1	1	1			
Sodium chlorid for 3 days																4	3			
Steer:																				
Untreated															4	3	2	2	2	1
Potassium chlorid for 1 day					4	3	3	2	2	2	2	2	1	1	1	1	1	1	1	1
Rubidium chlorid for 1 day								4	3	3	3	2	2	2	2	1	1	1	1	1
Lithium chlorid for 1 day																	4	3	3	2
Sodium chlorid for 1 day																4	3	2	2	1

from loss of sodium is likely to be masked by injury to the corpuscles, were incubated in sodium chlorid for three days. After the fragility had been increased as shown in the table, the corpuscles were transferred to a solution of potassium chlorid for two hours with the result recorded as the potassium effect.

In human corpuscles rubidium was found to be capable of replacing potassium in the production of an increase in fragility. This was tested by first incubating corpuscles in sodium chloride solution, thus giving them an opportunity to lose their potassium and increased resistance. They were then transferred to rubidium chlorid, in which they increased in fragility. To check against the possibility that the effect of rubidium might be merely destructive, the corpuscles were again placed in sodium chlorid; they were found to regain their increased resistance. A similar experiment was tried with lithium. Cat corpuscles were incubated for one day at room temperature in potassium chlorid to lose their sodium

TABLE 3

The ability of lithium to replace sodium in the production of an increase in fragility in cat corpuscles

CORPUSCLES INCUBATED IN	PER CENT										
	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48	0.51
Untreated							4	3	2	1	1
Potassium chlorid for 3 days at 22°C	4	3	2	1	1	1	1	1	1	1	1
Lithium chlorid for 1 hour				4	3	3	2	2	1	1	1
Potassium chlorid for 2 hours	4	2	2	1	1	1	1	1	1	1	1

and increase in resistance. They were then transferred to lithium chlorid for one hour, in which they increased in fragility. To differentiate against the toxic effect of lithium, they were returned to potassium chlorid, in which their original resistance was regained as the following data show (table 3). It would seem probable that lithium could replace sodium in producing an increase in fragility in those corpuscles which have a natural affinity for sodium as shown by their high sodium content, and that rubidium could replace potassium in producing increase in fragility in those corpuscles which have a natural high potassium content.

In view of the like behavior of rubidium and potassium, with reference to the production of changes in resistance, it was expected that cesium, because of a chemical similarity to these metals and because of a similar production of certain other biologic phenomena, would behave with reference to changes in resistance in the same manner as rubidium and potassium. Every opportunity was given for an increase in fragility of human corpuscles to occur in cesium chlorid. Corpuscles were incubated

for three days in an isotonic solution of that salt. This treatment caused an increase in resistance instead of an increase in fragility. They were also incubated in potassium chlorid for three days to insure the condition which might result in an increase in fragility, and were then transferred to cesium chlorid, in which, however, a marked increase in resistance occurred (table 4). Dog corpuscles incubated in cesium chlorid also increased in resistance. Corpuscles of both types increased in resistance in cesium chlorid (table 4).

The bivalent metals in all instances caused an increase in resistance (table 5).

TABLE 4

Comparison of the effect of cesium chlorid on the resistance of the corpuscles of man and dog

CORPUSCLES INCUBATED IN	READING OF RESISTANCE TEST, SODIUM CHLORID, PER CENT																				
	0.03	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48					
Human corpuscles in cesium chlorid																					
Untreated									4	2	1	1	1	1	0						
Incubated 3 days								4	2	2	1	1	1	1	1						
Incubated in potassium chlorid 3 days, transferred to cesium chlorid and kept in ice box for 3 days																					
Potassium chlorid															4	3					
Cesium chlorid				4	3	2	1	1	1	1	1	1	1	1	1	1					
Dog corpuscles in cesium chlorid																					
Untreated										4	3	1	1	1	1	0					
Cesium chlorid 1 day	4	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1					

Quantitative results were obtained after incubating dogs' blood in potassium and magnesium chlorids, and these were compared with the original content of the corpuscles. The methods used were the same as for human blood. A comparison of the metal contents and the changes in resistance follows (table 6). It will be seen that, with the increase in resistance on incubating in potassium chloride solution, there was a marked loss of sodium from the dog corpuscles, which was of the same degree as the loss of potassium which accompanied a similar increase in resistance of human corpuscles. This increase in resistance occurred in spite of a somewhat more than equivalent replacement of the original metal by potassium, for the value of the sodium in the untreated corpuscles was 8.6 per cent molar, while the molar value of the potassium

TABLE 5

Comparison of the effect of the bivalent metals, magnesium and calcium, on corpuscles having a high potassium content, with their effect on those with a high sodium content

CORPUSCLES INCUBATED IN	READING OF RESISTANCE TEST, SODIUM CHLORID, PER CENT																
	0.03	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48	0.51
Man:																	
Untreated											3	2	1	1	0		
Magnesium chlorid			4	3	2	2	1	1	1	1	1	1	1	1	1	1	1
Calcium chlorid	4	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Horse:																	
Untreated													4	3	3	3	2
Magnesium chlorid				4	3	2	2	2	1	1	1	1	1	1	1	1	1
Calcium chlorid					4	3	2	1	1	1	1	1	1	1	1	1	1
Rabbit:																	
Untreated													4	3	2	1	1
Magnesium chlorid				4	3	3	2	1	1	1	1	1	1	1	1	1	1
Calcium chlorid				4	3	2	1	1	1	1	1	1	1	1	1	1	1
Guinea pig:																	
Untreated											4	3	2	1	1	0	
Magnesium chlorid	4	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Calcium chlorid	4	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1
Dog:																	
Untreated										4	3	1	1	1	1	0	
Magnesium chlorid		4	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1
Calcium chlorid	4	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Cat:																	
Untreated										4	3	2	2	1	1	1	Trace
Magnesium chlorid				4	3	3	2	2	1	1	1	1	1	1	1	1	1
Calcium chlorid						4	2	2	1	1	1	1	1	1	1	1	1
Steer:																	
Untreated															4	3	2
Magnesium chlorid	4	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1
Calcium chlorid					4	3	3	3	2	2	2	2	1	1	1	1	1

in the potassium treated corpuscles was 9.1 per cent. It is very evident that in dog corpuscles resistance is a function of the sodium content. On the assumption that the metals occur as cations, it is independent of the total osmotic pressure of the combined metal contents, and potassium is passive in its production. With the entrance of a large amount of magnesium into the corpuscle, there was a tremendous increase in resistance which considerably outranked the increase on incubation in potassium. The magnesium content was 4.8 per cent molar, or half of that of the original sodium content. Its chemical equivalence was equal to that of the original sodium content. The blood of the steer was incubated in 5 cc. amounts for one day at 36°C. in sodium chlorid, and potassium chlorid.

TABLE 6

Comparison of the changes in metal content with the changes in resistance of dog corpuscles incubated one day in potassium and magnesium chloride solutions

CORPUSCLES INCUBATED IN	READING OF RESISTANCE TEST, SODIUM CHLORID, PER CENT																	MGV. FOR EACH 100 CC. CORPUSCLES		
	0.00	0.03	0.06	0.09	0.12	0.15	0.18	0.21	0.24	0.27	0.30	0.33	0.36	0.39	0.42	0.45	0.48	Sodium	Potassium	Magnesium
Untreated												4	3	2	1	1	0	198	32	6
Potassium chlorid				4	3	3	2	1	1	1	1	1	1	1	1	1	1	26	357	9
Magnesium chlorid	4	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		72	105

TABLE 7

Blood of steer incubated in isotonic solutions of sodium and potassium chlorid for one day; change in volume and change in sodium content

CORPUSCLES INCUBATED IN	VOLUME	SODIUM MGM. FOR EACH 100 CC.
Initial	3.80 cc. corpuscles	322 on basis of original volume
Sodium chlorid	3.52 cc. corpuscles	352 on basis of original volume
Potassium chlorid	3.90 cc. corpuscles	41 on basis of original volume

The results with respect to change in fragility in the corpuscles of the steer are given in table 2. As no hemolysis occurred in any of the preparations it was possible to note changes in volume. Sodium determinations were made. The results are recorded in table 7.

DISCUSSION AND CONCLUSIONS

It has been found that the corpuscles of man, rabbit, horse and guinea pig, which normally have a high potassium content, increase their fragility to hypotonic salt solution under conditions in which they are able to take up potassium or rubidium, and increase in resistance under condi-

tions in which they are enabled to lose potassium or rubidium, as on incubation in sodium or lithium chlorid. That the resistance increases notwithstanding the replacement of potassium by sodium has been previously shown by the quantitative determination of the metal content for one of these species of corpuscles, and it is inferred that in this group lithium is equally passive in the production of increases in fragility.

It has been found that the corpuscles of the cat, the dog, and the steer, whose metal content is preponderatingly sodium, increase in fragility under circumstances in which they are able to take up sodium or lithium, and increase in resistance under conditions in which they are enabled to lose sodium or lithium, as on incubation in rubidium or potassium chlorids. That resistance increases notwithstanding a replacement of sodium by potassium in molar equivalents has been established by quantitative determination of the metal content for the corpuscles of the dog.

The resistance to hypotonic salt solution, both in human corpuscles and in the corpuscles of the dog, would, on the assumption that the metals are present as cations seem to be unaffected by the osmotic pressure of the metal contents, as there is no relationship between the total molar value of the metal contents and resistance.

The results clearly show that the univalent metals, sodium and potassium, bear entirely different relationships to one physical property of the cell, namely, dissolution by water. Since the effects of these metals are reversed in the two types of corpuscles studied, this respective relationship can not depend on the individual properties of the metals, but must depend on a mutual relationship between the properties of the corpuscle substances and the properties of the cations; and since the two elements which are chemically like sodium and potassium respectively align with them in these relationships, it would seem likely that this interrelationship is chemical.

It is evident that any property of the ion inherent to its degree of hydration, as determined by its migration velocity, which lately has been of interest in the attempt to find the explanation of the wonted presence of potassium in plant and animal cells in excess of that in their environment, is not a factor in this change of resistance, since sodium, which has a comparatively low migration velocity, will produce the same effect as potassium, which has a comparatively high migration velocity, if the type of corpuscle with which it is allowed to react is appropriately selected.

Since sodium in the cells of the cat, dog, and the steer has shown the same apparent causal relationship to change of dissolubility of the corpuscle by hypotonic salt solution that potassium has shown to cells in which it is in preponderance, it would not seem probable that sodium is in these cells merely as a result of its presence in the serum, but that it is present in the corpuscles of these animals because it bears the same rela-

tionship to their cell substance that potassium does to the corpuscle substance of those cells in which that metal is in preponderance; and as sodium does not bear this relationship because of any greater hydration of the ion, since the hydration is not greater, it would seem likely that in the other type of corpuscle, potassium also is not present in excess because of this specific property of the potassium ion, but because of a specific mutual relationship between the ion and the cell substance. Further, if the hydration of the ion should not explain the preponderance of potassium in the red corpuscle, this may not be the explanation of the preponderance of potassium in other cells.

The water content of the corpuscle as indicated by change in volume does not necessarily parallel changes in resistance. Although human corpuscles kept at body temperature increased in volume with the gain in potassium, resulting in greater fragility, and decreased in volume when they lost potassium and increased in resistance, the corpuscles of the steer lost in volume when they increased their fragility in sodium chloride solution and gained in volume when they increased in resistance on incubation in potassium chloride solution.

With the bivalent metals, magnesium and calcium, an increase in resistance occurs in all instances of both types of corpuscles incubated in isotonic solutions of their chlorids.

BIBLIOGRAPHY

(1) Ashby: This Journal, 1924, lxviii, 239.
(2) Ashby: This Journal, 1924, lxviii, 250.
(3) Bayliss: Principles of general physiology. 2nd ed. New York. 1918.
(4) Höber: Biochem., Zeitschr., 1908, xiv, 209.
(5) Höber: 5th ed. Leipsig, 1922.
(6) Loeb: Journ. Gen. Physiol., 1920, ii, 673.
(7) Mitchell and Stanton: Journ. Gen. Physiol., 1921–1922, iv, 141.

STUDIES ON CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XII. Changes in Metabolism Following Adrenal Stimulation

MONROE A. McIVER[1] AND ELIZABETH M. BRIGHT

From the Laboratories of Physiology of the Harvard Medical School

Received for publication February 25, 1924

Evidence of the important influence exerted by the adrenal glands on metabolism has been accumulating for a number of years. Since the subject is complicated, it is fortunate that various methods have been used in the several lines of work directed toward the solution of this problem. Studies on the changes in metabolism caused by removal of the adrenals, and observations on the effects of injecting adrenalin into the intact animal or of treating organs or tissues with the drug, have furnished us with many data. In reporting the present research it seems unnecessary to summarize the literature, since the recent contribution by Boothby (1) carries with it a complete review of the publications on the subject.

There is nearly universal agreement that stimulation of a splanchnic nerve evokes adrenal secretion. The work of Cannon and his co-workers (2), (6) has demonstrated that stimulation of a sensory nerve likewise will cause a marked liberation of adrenin. These facts were used in the following studies, in which a supply of adrenin was required under conditions as nearly physiological as possible as regards dosage, passage of the secretion into the circulation, and minimum disturbance of the other functions of these compound glands.

Method. *Standard metabolism.* In all the experiments recorded in this paper, before stimulation of the adrenals, the metabolic level was accurately determined, often over several periods. To obtain a uniform series of experiments, it is desirable that the preliminary metabolic readings should represent the true "basal," or, in Krogh's more satisfactory term (3), the true "standard" metabolism. Rigid requirements are necessary to insure standard metabolic activity; and in our experiments the problem was complicated by the effects of the anesthetic,—a point discussed below. Standard conditions were carefully observed, as follows. The animals were given no food for from eighteen to twenty-four hours prior to the experiments. Before the preliminary readings, ample time was allowed for the subsidence of the stimulation incident to anesthetization or resulting

[1] Medical Fellow of the National Research Council.

from adrenin thrown into the circulation by operative procedures. In the earlier experiments the importance of the length of this period was not sufficiently appreciated; in the later work a two-hour interval following completion of all operative procedures proved to be an adequate period for the return of the metabolism to a condition of minimum activity.

Anesthesia; effect of urethane. The experiments recorded in this paper were all carried out under urethane anesthesia. The use of ether was considered, but was rejected because it interfered with accurate gas analysis. Other non-volatile anesthetics were not employed, since, for the sake of comparison, a single anesthetic in all the experiments was advantageous. Four cubic centimeters of a 25 per cent solution of urethane per kilo of body weight were given as a basic dose, although in most cases a small additional amount was required before a satisfactory grade of anesthesia was obtained. Except in a few instances, indicated in the protocols, the urethane was given intravenously. By using a small amount of ethyl chloride as a local anesthetic, the femoral vein was quickly and easily exposed and furnished a satisfactory route for administration. This method of giving the anesthetic has distinct advantages over administration by stomach tube, for smaller doses can be given, the stage of excitement is minimized, and the depth of anesthesia can be much more accurately regulated.

Aub, Bright and Uridil (4) found that when urethane was administered the metabolism of the cats used in their control experiments showed an average rise of 15 per cent, and they are inclined to attribute this increased activity to the effect of urethane upon the adrenals. In the same paper they give the average "basal" metabolism of 54 unanesthetized cats as 2.29 calories per kilo of body weight, and the average body temperature as 39.2. In our experiments the average standard metabolism, under urethane anesthesia, of 10 cats in which no preliminary operative procedure had been carried out was 2.71 calories per kilo, with an average body temperature of 37.2. This difference of 18 per cent in metabolism between the unanesthetized and the anesthetized cats may be attributed to the effects of urethane.

In a second paper (5), Aub and his co-workers report that removal of the adrenals in urethanized cats results in a prompt drop of 12 per cent in metabolism, and they present evidence for thinking that there is a greater flow of adrenin under urethane anesthesia than there is normally. This was somewhat higher than our figures in 17 cases in which the adrenals were removed for purposes of control, where the fall in metabolism averaged 7 per cent. Our lower average figure may be explained by the fact that in a number of instances the adrenals were denervated in a preliminary operation. There were four instances (experiments 8, 10, 15 and 28) in which one or more metabolic determinations after removal of the adre-

nals showed a higher metabolic rate; but it must be remembered that the adrenals had been stimulated a short time before the operation was performed, and it is possible that the metabolic activity from this had not had sufficient time to subside.

Since the evidence at hand is in favor of a stimulation of the adrenals by urethane, it would obviously be of advantage to eliminate this factor before determining the standard metabolism of the animals. To do this, the expedient was tried of removing one adrenal and cutting the splanchnic nerve supplying the remaining gland. This procedure leaves one gland in situ, properly supplied with blood, but removed from excitation by the central nervous system. In this type of experiment the gland was activated by stimulating the distal end of the cut splanchnic. The procedure of removing the adrenals from nervous control was carried out at the beginning of the experiment with the belief that the stimulating effect of urethane upon the adrenals was due primarily to the effect of this drug upon the central nervous system; and the results obtained confirmed this view, for the metabolism of the animals in which the adrenals had been denervated was much lower, averaging only 2.12 calories per kilo. This is 21.6 per cent lower than in animals on which this operation had not been performed, and 7.5 per cent lower than the figures given by Aub and his co-workers for normal unanesthetized cats. The denervation of the adrenals was performed in the later experiments, and we consider it very important in obtaining uniform standard conditions under urethane anesthesia. It will be noted on consulting table 1 that in 5 cases (experiments 5, 6, 10, 12 and 22) no increase in metabolism followed adrenal stimulation. All these were cases in which this preliminary denervation of the adrenals had not been carried out.

Studies were carried out to determine the relation between the depth of anesthesia and the metabolic rate. The data obtained indicated that the rate of metabolism was not greatly altered by changes, within reasonable limits, in the depth of anesthesia. Thus, in experiments 11, 21 and 22 (table 1), the dose was increased 0.48, 0.15 and 4.00 cubic centimeters per kilo, respectively, but with little effect on the metabolic rate, except by the massive dose of 4 cubic centimeters per kilo given in experiment 22. Taking all the experiments into consideration, there seems to be a gradual slowing of metabolism under urethane anesthesia; but this may be due to a gradual subsidence of the excitation incident to anesthetization and the preliminary operative procedures, rather than to the simple effect of the anesthetic.

Modes of adrenal stimulation. While in all the experiments the adrenals were activated by nervous stimulation, a number of different methods were attempted before a standard technic was adopted. The sciatic, brachial and splanchnic nerves were stimulated at different times, but splanchnic

stimulation was finally decided upon as giving the best results. The nerve, after exposure by the dorsal route, was ligated and cut; the distal end was then stimulated by a tetanizing current, turned on and off every second by an interrupter. An approximately uniform strength of current was used

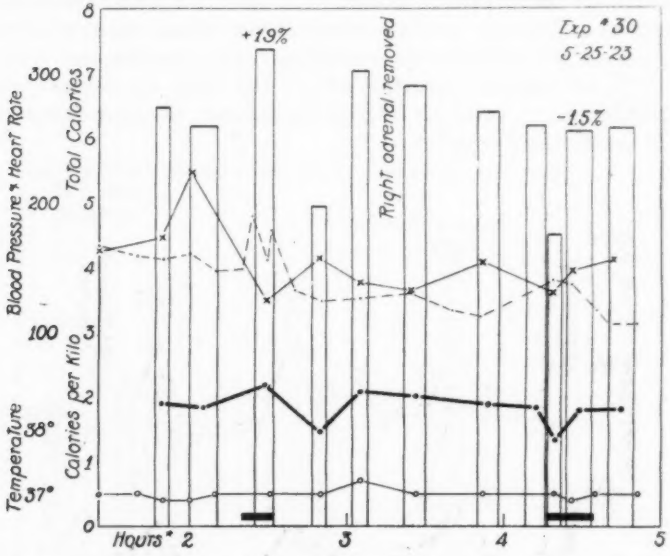


Fig. 1. Showing the rise in metabolism produced by activating the right adrenal by means of splanchnic stimulation.

A repetition of the stimulus after removal of the adrenal failed to increase the metabolic rate.

Preliminary operation: Thyroid removed, left adrenal removed. The nerves to the liver cut and the right splanchnic nerve prepared for stimulation.

In this and in the following figures the height of the rectangles represents the metabolic activity in terms of total calories per hour. The percentage of variation occurring during the stimulation is written above. A heavy solid line at the bottom of a rectangle represents a stimulation period.

o——o Body temperature.
•——• Calories per kilo per hour.
x——x Blood pressure.
· · · · · · · Heart rate.
* Number of hours after the completion of operative procedures.

in all the work, care being taken to select a strength which did not spread to surrounding tissues. In a number of the earlier experiments the heart was denervated according to the technic used by Cannon (6). This preparation revealed whether an adequate liberation of adrenin occurred when the stimulation was applied. In later work this procedure was largely discontinued, because stimulation so uniformly resulted in adrenin secretion.

Final type of experiment. Experiment 30, figure 1, represents a complete experiment carried out according to the technic finally adopted. Effort was made to reduce the experiment to the simplest terms, so that the effects of stimulation would be confined as far as possible to the adrenal gland. A preliminary operation was performed in which the thyroid was removed in order to exclude any possible action of that gland; the hepatic nerves were cut, to prevent splanchnic impulses from reaching the liver; and finally the left adrenal was removed and the right splanchnic prepared for stimulation. A two-hour interval was allowed to elapse before collection of the standard samples.

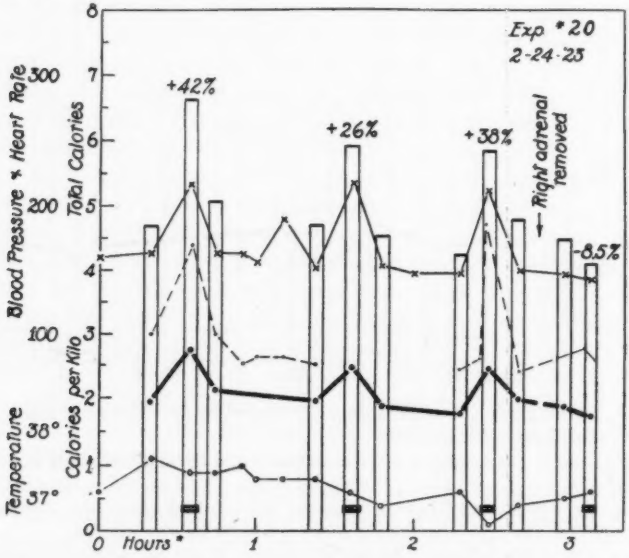


Fig. 2. Repeated increase in the metabolic rate obtained by splanchnic stimulation. Note the failure to obtain a rise after the remaining adrenal had been removed. Preliminary operation: The heart denervated, the left adrenal removed and the right splanchnic nerve prepared for stimulation.

The experiment consisted, briefly, in an estimation of the metabolism before, during and after nervous stimulation of the adrenal under standard conditions. The work was controlled by repeating the stimulation and metabolic determinations after removal of the adrenal, the other conditions remaining the same. It will be observed that stimulation of the distal end of the divided splanchnic resulted in a 19 per cent increase of metabolism. No increase of metabolism resulted from the second stimulation, although the conditions were the same in the first and second except for the presence or absence of the adrenal. The body temperature was recorded by a rectal

thermometer and a constant level was maintained by supplying heat, when necessary, from an electric pad. In every case the heart rate and blood-pressure changes were followed by means of a mercury manometer connected with a cannula placed in the carotid or femoral artery. In certain instances the respiratory rate and depth were also recorded by means of a tambour attached around the thorax.

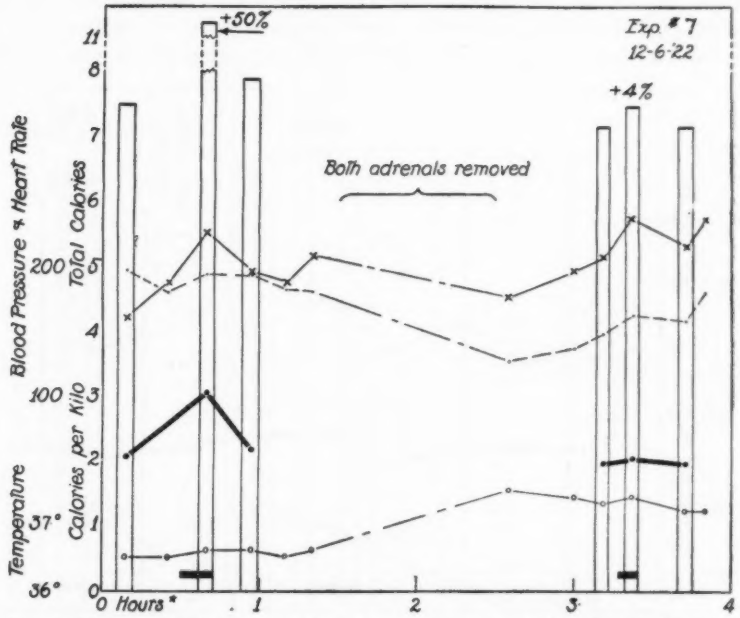


Fig. 3. Rise in metabolism resulting from activation of the adrenals by sciatic stimulation. The slight increase in metabolism obtained on repeating the stimulation after removal of the adrenals probably due to the fact that the hepatic nerves had not been cut.

Preliminary operation: The thyroid removed. The sciatic nerve prepared for stimulation.

The course of the metabolism was followed by determinations of the gaseous exchange. By means of a T-shaped cannula fitted with Thiry-Tissot valves, the expired air was collected, over periods of from five to ten minutes' duration, in two 8-liter copper spirometers. The samples for analysis were then collected over mercury and analyzed with the portable type of Haldane apparatus. The analyses were made in duplicate and routine determinations of room air were carried out as checks. The calories have been calculated on the basis of oxygen consumption; the metabolism is stated in total calories per hour and also in calories per kilo per hour in

figures 1 to 5. In table 1 the results are given only in calories per kilo per hour.

Controls. In removing the adrenals for purposes of control, the dorsal route was always employed. The technic is simple; the operation can be carried out in about ten minutes and involves no loss of blood or interference with the abdominal viscera or blood-pressure changes. After this operation the metabolic level was again determined and the stimulation repeated. Although there were several experiments, all of which were carried out without the preliminary precaution of cutting the hepatic nerves,

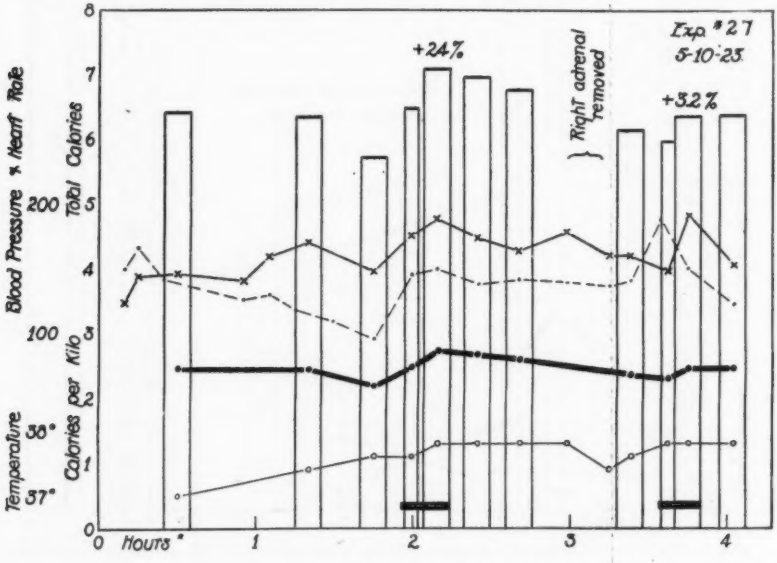


Fig. 4. The same type of experiment as shown in figure 1, except for the fact that the nerves to the liver were not cut in this instance.

Preliminary operation: Removal of the thyroid and left adrenal. The right splanchnic nerve prepared for stimulation.

in which slight rise was recorded after removal of the adrenals, in only two instances (experiments 26 and 29, table 1) was this comparable with that which occurred when the adrenals were present (see also figs. 3 and 4). That splanchnic stimulation is sometimes able to cause an increase in metabolism after removal of the adrenals is shown in experiment 32, table 1. It will be noted that this increase did not occur when stimulation was applied after hepatic nerves had been cut.

Experiments 9 (fig. 5) and 31 (table 1) were carried out as special controls. In these experiments the adrenals were removed as a preliminary

step and the splanchnic was then stimulated, after a delay comparable to that allowed when the adrenals were present.

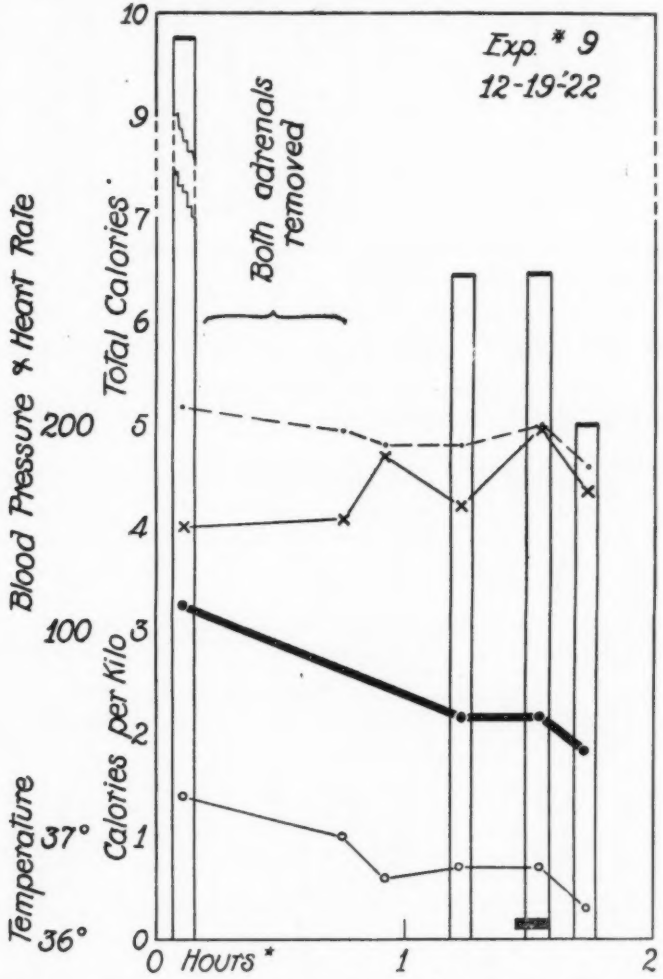


Fig. 5. This experiment was carried out as a special control. The stimulation period before removal of the adrenals was omitted in order that the length of time elapsing between the administration of the anesthetic and the stimulation period should be comparable to the time interval in those cases in which the adrenals were present. It will be noted that no increase in metabolism occurred upon stimulation.

Preliminary operation: Removal of the adrenals and preparation of the sciatic nerve for stimulation.

TABLE 1

EXPERIMENT NUMBER, DATE, WEIGHT, PRELIMINARY OPERATION	STIMULATION	TIME	CALORIES PER KILO	VARIATION	R.Q.	VENTILATION	PULSE	TEMPERATURE	REMARKS
				per cent		*cc./min.*		*°C.*	
1		11:00	2.21		0.79	635	176	37.4	It will be noted that the first three experiments given in this table were not controlled by removal of the adrenals
10–24–22		1:25	2.10		0.78	573	176	37.3	
3.00 kg.	1:49–1:59	1:49	2.37	+13.0	0.93	881	212	37.6	
Heart denervated	Sciatic stimulation	2:07	2.26		0.77	559	180	37.8	
Sciatic exposed		2:33	2.29		0.84	629	203	37.9	
10:45 Preparation complete									
2		10:53	2.55		0.74	791	232	38.3	
11–6–22	11:12–11:23	11:13	2.46	−3.6	0.80	691	244	38.3	
4.2 kg.	Brachial stimulation	11:45	2.47		0.86	770	248	38.3	
Heart denervated		12:25	1.73		0.84	540	188	38.5	12:00 Left splanchnic cut and prepared for stimulation. Right adrenal tied off
Brachial exposed	12:44–12:54	12.45	2.63	+52.0	0.87	825	276	38.4	
10:30 Preparation complete	Splanchnic stimulation	1.05	1.54		0.86	498	188	38.5	After failure of brachial stimulation the adrenals were removed from the influence of the anesthetic; after this procedure splanchnic stimulation resulted in a marked increase in metabolism
3		11:29	1.68		0.71	516	192	36.0	In this experiment both types of stimulation resulted in an increased metabolism
11–9–22	11:54–12:07	11:55	2.07	+23.0	0.79	668	196	36.3	
3.1 kg.	Brachial stimulation	12:03	1.92		0.83	659	212	36.2	
Heart denervated		12:22	1.73		0.73	561	181	36.2	
Brachial exposed		12:48	1.46		0.81	550	181	36.5	12:35 Left splanchnic cut. Blood pressure low during latter part of the experiment
11:00 Preparation complete	1:01–1:08 Splanchnic stimulation	1:03	1.69	+16.0	0.81	574	196	36.1	

4		11:28	2.93		0.72	1160	210	37.3	
11:20:22	11:42–11:47	11:42	3.27	+11.5	0.97	1360	244	37.2	
3.9 kg.	Splanchnic	11:57	2.97		0.87	1333	214	37.0	12:50 Right and left adrenals removed
Heart denervated	stimulation	12:51	2.58		0.85	1160	180	37.3	
Rt. Splanchnic cut	1:07–1:16	1:10	2.47	−4.4	0.86	1090	180	37.3	First experiment controlled by removal of the adrenals
10:30 Preparation complete	Splanchnic stimulation	1:26	2.47		0.84	1070	180	37.4	
5		11:27	2.75		0.75	656	199	37.8	
11–22–22	11:49–11:55	11:50	2.73	−14.0	0.95	607	244	37.3	
2.4 kg.	Splanchnic	12:04	2.68		0.76	677	192	37.2	12:20 Adrenals removed
Heart denervated	stimulation	2:10	2.65		0.78	700	163	37.2	
Rt. Splanchnic cut	2:25–2:32	2:25	2.60	−2.0	0.78	650	162	37.0	One of the experiments that failed to show a rise during adrenal stimulation. The high initial metabolism probably due to action of the anesthetic
11:10 Preparation complete	Splanchnic stimulation	2:46	2.37		0.81	628	156	36.8	
6		10:33	2.93		0.66	1241	180	36.8	
12–4–22	10:51–11:00	10:52	2.54	−6.0	0.82	1308	197	36.9	Another experiment failing to show an increase upon stimulation. Note the high initial metabolic level
2.4 kg.	Sciatic stimulation	10:58	2.87		0.92	1783	220	36.9	
Sciatic exposed		11:08	2.69		0.76	1363	208	36.9	
Heart not denervated									
10:20 Preparation complete		12:39	2.18		0.75	1343	240	36.7	12:05 Both adrenals removed
	12:51–12:58	12:52	1.95	−10.5	0.71	1070	258	37.0	
	Sciatic stimulation	1:04	1.93		0.58	1028	220	37.1	
7		10:36	2.02		0.77	694	160	36.5	
12–6–22	11:00–11:13	11:07	3.03	+50.0	0.83	2221	225	36.6	See figure 3
3.7 kg.	Sciatic stimulation	11:23	2.13		0.73	800	196	36.6	
Thyroid removed		1:38	1.93		0.74	784	207	37.3	12:00–1:00 Both adrenals removed
Heart not denervated	1:47–1:54	1:49	2.01	+4.0	0.72	861	236	37.4	
10:31 Preparation complete	Sciatic stimulation	2:09	1.93		0.73	703	213	37.2	

TABLE 1 —*Continued*

EXPERIMENT NUMBER, DATE, WEIGHT, PRELIMINARY OPERATION	STIMULATION	TIME	CALORIES PER KILO	VARIATION	R.Q.	VENTILATION	PULSE	TEMPERATURE	REMARKS
				per cent		*cc./min.*		*°C.*	
8		11:52	2.92		0.81	2155	184	36.7	
12–8–22	12:03–12:07	12:03	3.96	+35.5	0.94	2920	246	36.7	
3.6 kg.	Sciatic stimulation	12:15	2.53		0.80	1059	216	36.5	12:22–12:52 Both adrenals removed
Thyroid removed		1.34	3.15		0.75	2570	166	36.7	
Sciatic exposed	1:43–1:53	1:44	3.13	−0.6	0.73	2300	240	36.5	Metabolism high after removal of both adrenals
10:50 Preparation complete	Sciatic stimulation	1:58	2.25		0.84	2184	156	36.5	
9		11:48	3.29		0.85	1218	150	37.4	11:56–12:30 Both adrenals removed
12–19–22		12:56	2.15		0.74	1127	160	36.7	1:00 Sciatic isolated
3 kg.	1:12–1:18	1:14	2.16	±	0.76	862	199	36.7	Special control shown in figure 5
See "Remarks"	Sciatic stimulation	1:26	1.83		0.75	679	168	36.3	
10		10:12	2.73		0.78	1560	148	36.9	Slight twitching at times during both stimulation periods. Fall in blood pressure during first sciatic stimulation
12–21–22	10:38–10:57	10:44	2.29	−16.0	0.89	1590	188	36.8	
3.6 kg.	Sciatic stimulation	10:56	2.17		0.75	900	192	36.8	
Thyroid removed									
Sciatic isolated		11:43	2.40		0.78	1704	160	36.5	11:05 Both adrenals removed
9:35 Preparation complete	11:57–12:04	11:58	2.07	−13.6	0.79	2126	196	36.6	Animal deeply narcotized
	Sciatic stimulation	12:11	1.78		0.83	2005	179	36.6	
11		12:22	2.10		0.77	833		36.5	11:58 1 cc. urethane
1–11–23	12:31–12:36	12:34	2.22	+5.8	0.80	968	200	36.5	12:21 1 cc. urethane
4.2 kg.	Sciatic stimulation	12:44	2.25		0.72	888	196	36.5	12:55 2 cc. urethane
Heart denervated		12:59	2.19		0.74	865	196	36.6	1:24 2 cc. urethane
10:20 Preparation complete		1:26	2.04		0.74	725	194	36.6	Urethane was given in addition to a basic dose of 4.7 cc. per kilo
	1:36–1:41	1:37	2.60	+27.2	0.86	1121	204	36.7	Experiment to test the effect of increased doses of urethane
	Sciatic stimulation	1:46	2.02		0.74	726		36.7	

12		1:22	2.64		0.75	535	202	37.2	A preliminary denervation of the adrenals should have been carried out
1–15–23	1:33–1:40	1:35	2.64	±	0.87	733	246	37.2	
2.3 kg.	Sciatic stimulation	1:46	2.15		0.81	493	220	37.2	
Heart denervated									
Sciatic isolated									
12:00 Preparation complete									
13		11:28	2.29		0.81	459	200	37.4	
1–19–23	12:07–12:28	12:07	2.63	+15.0	0.81	526	212	37.1	12:19–12:27 Injection of 20 cc. 5% glucose solution
2.6 kg.	Splanchnic	12:20	2.33		0.79	445	196	37.1	
Heart denervated	stimulation	12:32	2.23		0.76	450	192	37.3	12:15 Injection of 15 cc. 5% glucose solution
Right splanchnic exposed		1:56	1.96		0.75	377	182	37.5	
		2:24	2.08		0.79	396	176	37.2	It is apparent that injection of the glucose had little effect on the results of splanchnic stimulation
10:25 Preparation complete	2:34–2:42	2.35	2.35	+20.0	0.94	495	205	37.2	
	Splanchnic stimulation	2:50	2.23		0.75	397	176	37.3	
14		11:42	2.53		0.72	2061	200	37.4	
1–23–23	11:58–12:11	12:05	2.29	−9.5	0.84	1631	223	37.3	
3.2 kg.	Splanchnic	12:22	2.62		0.65	1276	198	37.3	12:33–12:40 Intravenous injection of glycocoll—3.2 grams in 25 cc. distilled H_2O (1 gram per kilo)
Heart denervated	stimulation	1:17	2.38		0.83	1265	206	37.4	
Splanchnic exposed	1:29–1:40	1:31	2.51	+5.2	0.82	1108	224	37.5	
11:35 Preparation complete	Splanchnic stimulation	1:45	2.90		0.78	1356	206	37.4	Glycocoll also did not greatly influence the effects of stimulation
15		10:48	2.74		0.75	1393	168	37.8	
1–25–23	11:05–11:14	11:06	2.81	+1.6	0.79	1213	214	37.8	
3.6 kg.	Splanchnic	11:18	2.66		0.83	1442	174	37.8	11:45–11:47 3.6 gr. glycocoll in 40 cc. dist. H_2O injected into loop of small intestine
Heart denervated	stimulation	12:38	2.85		0.81	2021	172	38.0	
Splanchnic isolated	12:52–12:02	12:54	2.97	+4.0	0.88	1519	188	37.8	
10:35 Preparation complete	Splanchnic stimulation	1:05	2.76		0.86	1385	168	37.5	1:17–1:42 Adrenals removed
		1:46	2.89		0.84	2022	156	38.2	
	1:58–2:09	2:01	2.65	−8.4	0.83	997	152	38.1	
	Splanchnic stimulation	2:13	2.59		0.80	1033	154	38.1	

TABLE 1—*Continued*

EXPERIMENT NUMBER, DATE, WEIGHT, PRELIMINARY OPERATION	STIMULATION	TIME	CALORIES PER KILO	VARIATION	R.Q.	VENTILATION	PULSE	TEMPERATURE	REMARKS
				per cent		*cc./min.*		*°C.*	
16		11:05	2.34		0.80	496	212	39.0	
1–29–23	11:34–11:46	11:36	2.89	+23.0	0.84	588	256	39.0	
2.8 kg.	Splanchnic	11:50	2.42		0.77	524	198	39.0	
Heart denervated	stimulation								
Splanchnic isolated									
10:50 Preparation complete									
17		1:54	2.40		0.63	388	136	37.0	Note that the preliminary operation removing the adrenals from the effect of the anesthetic was carried out the preceding day
2–9–23	2:18–2:28	2:18	2.89	+20.0	0.88	616	188	37.1	
2.8 kg.	Splanchnic	2:35	2.56		0.76	513	144	37.1	
Heart denervated	stimulation								
Right splanchnic cut									
Left adrenal removed preceding day									
1:15 Preparation complete									
18		4:23	3.32		0.66	415	220	38.0	
2–12–23	4:37–4:41	4:37	3.13	−5.6	0.98	406	256	37.8	
2 kg.	Splanchnic	4:45	3.08		0.75	435	220	38.2	
Heart denervated	stimulation	5:08	2.88		0.76	460	218	38.3	5:30–5:44 Coeliac axis ligated. Superior mesenteric artery ligated. Aorta ligated below kidneys. Liver ligated by lobes. Although the liver was removed the secretion from the right adrenal could still reach the upper extremity
Left adrenal removed	5:18–5:23	5:20	3.06	+6.0	0.93	566	268	38.3	
Right splanchnic cut	Splanchnic	5:29	2.87		0.82	519	216	38.2	
3:55 Preparation complete	stimulation	5:45	1.30		0.78	221	234	38.0	
	5:56–6:03	5:58	1.68	+29.0	0.89	283	268	38.2	
	Splanchnic stimulation	6:08	1.50		0.89	267	226	38.2	

	stimulation								the upper extremity
19		1:52	2.13		0.71	1169	172	36.9	
2–16–23	2:11–2:20	2:15	1.80	−15.5	1.00	1249	212	36.7	
3.00 kg.	Splanchnic	2:23	2.00		0.81	1008	184	36.9	Second stimulation produced a slight rise
Heart denervated	stimulation								
Left adrenal removed, right denervated.	2:46–2:52	2:47	2.12	+6.0	0.90	1150	198	36.6	
1:00 Preparation complete	Splanchnic stimulation	2:58	2.08		0.89	1520	176	36.8	
20		10:17	1.94		0.74	556	164	37.6	
2–24–23	10:32–10:38	10:32	2.76	+42.0	0.84	635	216	37.4	See figure 2
2.4 kg.	Splanchnic	10:41	2.11		0.81	573	164	37.4	
Heart denervated	stimulation	11:20	1.96		0.68	558	152	37.3	
One adrenal removed	11:32–11:39	11:33	2.47	+26.0	0.76	583	217	37.1	
Splanchnic exposed	Splanchnic	11:45	1.89		0.84	619	154	36.9	
10:00 Preparation complete	stimulation	12:15	1.77		0.76	509	148	37.1	
	12:26–12:31	12:26	2.44	+38.0	0.95	643	212	36.6	
	Splanchnic	12:37	1.99		0.77	578	150	36.9	12:42–12:52 Remaining adrenal removed
	stimulation	12:54	1.87		0.72	501	148	37.0	
	1:04–1:09	1:05	1.71	−8.5	0.75	453	144	37.1	
	Splanchnic stimulation								
21		11:00	1.96		0.82	850	196	36.2	10:52 10 cc. urethane
3–22–23		11:10	2.78		0.76	1228	210	36.3	
4.2 kg.	11:35–11:45	11:36	2.52	−9.4	0.85	122	190	37.0	
Left splanchnic exposed, ligated and cut	Splanchnic	11:51	2.56		0.83	990	188	37.0	12:02 3.5 cc. urethane
10:30 Preparation complete	stimulation	12:34	2.32		0.79	948	198	37.1	
	12:49–12:52	12:50	2.49	+7.2	1.01	1176	222	37.0	1:00–1:30 Both adrenals removed
	Splanchnic stimulation								
	1:46–1:52	1:01	2.21		0.81	998	192	36.9	
	Splanchnic	1:34	2.09		0.84	994	192	36.6	
	stimulation	1:37	2.04	−2.5	0.88	1011	216	36.5	

TABLE 1—*Continued*

EXPERIMENT NUMBER, DATE, WEIGHT, PRELIMINARY OPERATION	STIMULATION	TIME	CALORIES PER KILO	VARIATION	R.Q.	VENTILATION	PULSE	TEMPERATURE	REMARKS
				per cent		*cc./min.*		*°C.*	
22		1:34	3 33		0 64	3118		39.0	12:40 Intravenous injection of
4-4-23		1:48	3.10		0.72	2976	224	38.4	5.1 cc. of urethane per kilo of
4.2 kg.	2:00–2:19	2:00	2.98	−4.0	0.97	3198	216	38.0	body weight
Splanchnic exposed, ligated and cut	Splanchnic stimulation	2:15	3.05		0.86	3615	228	38.7	
		2:34	2.78		0.79	3560		39.0	2:45–3:02 4 cc. urethane per kg.
1:30 Preparation complete		3:09	2.43		0.80	1646	224	38.9	injected
		3:23	2.65		0.78	2058		38.9	
	3:36–3:42	3:37	2.62	−1.0	0.91	2269	248	39.0	
	Splanchnic stimulation	3:48	2.55		0.87	2325	244	39.0	4:02 5 cc. urethane injected.
		4:17	2.33		0.96	2204		39.0	
	4:30–4:34 Splanchnic stimulation	4:30	2.08	−10.6	1.06	2300	232	39.0	Massive doses of urethane
23		11:04	3.22		0.89	1300	218	37.6	
4-9-23		11:12	3.13		0.82	1234	208	37.6	
2.65 kg.	11:29–11:40	11:29	3.08		0.87	1144	204	37.5	
Right splanchnic exposed	Splanchnic stimulation	11:35	3.72	+19.0	0.90	1687	224	37.7	
		11:46	3.40		0.76	1591	200	38.0	12:04 Left adrenal removed.
10:50 Preparation complete		12:19	3.10		0.70	1303	232	37.7	Right adrenal undisturbed
		12:29	3.18		0.78	1488	224	38.0	
	12:44–12:53	12:44	2.89		0.95	1157	228	37.7	
	Splanchnic stimulation	12:50	3.22	+1.2	0.95	1390	216	37.7	1:20 6 cc. urethane injected
		1:38	2.79		0.78	672	202	38.0	
	1:54–2:02	1:55	3.09	+11.0	1.00	844	244	38.0	
	Splanchnic stimulation	2:05	3.14		0.87	924	248	38.0	
24		12:47	2.50		0.73	1008		38.2	

24		12:47	2.50		0.73	1008		38.2	
4-24-23		12:58	2.48		0.75	1021	113	38.2	Urethane in this experiment was given by mouth
3.5 kg.	1:12-1:30	1:16	2.73	+10.0	0.92	1093	140	38.2	
Splanchnic exposed, ligated and cut	Splanchnic stimulation	1:25	2.75		0.90	1109	139	38.2	
12:54 Preparation complete		1:37	2.55		0.79	1007	122	38.3	
25		3:40	2.36		0.75	675	196	36.2	
4-25-23	3:55-4:07	3:55	1.64		0.86	487	184	36.3	
2.88 kg.	Splanchnic	4:01	2.51	+7.0	0.88	751	216	36.5	4:12 Slight movement. Note the marked depression of metabolism on starting the stimulation. There was an accompanying depression of respiration
Splanchnic exposed, ligated and cut	stimulation	4:14	2.31		0.70	583	190	36.6	
3:20 Preparation complete									
26		2:10	1.56		0.76	818	134	36.9	
5-2-23		2:17	1.53		0.78	716	141	36.8	
3.1 kg.	2:28-2:56	2:28	1.74	+14.2	0.95	870	180	36.9	Stimulation for 28 minutes, the metabolism reaching its highest level after 6 minutes
Thyroid removed, left adrenal removed and right splanchnic cut	Splanchnic stimulation	2:34	1.79	+17.0	0.90	935	188	36.8	
		2:47	1.68		0.92	1280	167	36.8	
		3:36	1.62		0.70	1375	184	36.7	3:50-3:59 Remaining adrenal removed
1:40 Preparation complete		4:10	1.42		0.72	1556	148	37.0	
	4:27-4:41 Splanchnic stimulation	4:32	1.53	+8.0	0.78	1391	164	36.3	
27		10:10	2.47		0.83	795	143	37.0	
5-10-23		11:00	2.44		0.74	670	170	37.4	
2.6 kg.		11:25	2.20		0.83	720	148	37.6	
Thyroid removed, left adrenal removed, right denervated	11:41-11:56	11:42	2.49		0.99	867	176	37.6	
	Splanchnic stimulation	11:48	2.73	+24.0	0.92	969	188	37.8	See figure 4
		12:04	2.68		0.80	838	174	37.8	
		12:21	2.60		0.82	878	164	37.8	
9:47 Preparation complete		1:03	2.36		0.81	873	160	37.6	12:44-12:57 Remaining adrenal removed
	1:19-1:34	1:20	2.30		0.85	868	148	37.8	
	Splanchnic	1:25	2.44	+3.2	0.73	807	190	37.8	
	stimulation	1:42	2.44		0.78	950	152	37.8	

TABLE 1—*Continued*

EXPERIMENT NUMBER, DATE, WEIGHT, PRELIMINARY OPERATION	STIMULATION	TIME	CALORIES PER KILO	VARIATION	R.Q.	VENTILATION	PULSE	TEMPERATURE	REMARKS
				per cent		*cc./min.*		°C.	
28		12:58	2.07		0.65	845	172	37.0	
5–16–23		2:39	1.99		0.77	907	208	37.0	The animal was quite excited during the administration of the urethane
2.6 kg.		2:58	1.93		0.78	1081	204	37.0	
Thyroid removed		3:14	1.99		0.74	1020	204	37.0	
One adrenal removed and one denervated	3:29–3:45	3:34	2.19	+10.0	0.76	1051	220	36.6	
12:41 Preparation complete	Splanchnic stimulation	4:00	1.91		0.80	854	200	36.9	
		4:15	1.85		0.74	793	192	36.8	4:30 Remaining adrenal removed
		4:36	1.97		0.72	908	216	36.7	
		5:10	1.97		0.73	1049	228	36.9	
	5:26–5:41	5:31	1.92	−2.5	0.72	1010	216	37.0	
	Splanchnic stimulation	5:46	1.79		0.77	1094	208	37.0	
29		1:27	2.24		0.72	671	168	36.3	Eight per cent rise on stimulation after removal of the adrenals. Hepatic nerves intact
5–21–23		1:53	2.06		0.77	669		36.5	
2.7 kg.	2:11–2:25	2:16	2.56	+24.0	0.85	627	172	36.5	
Thyroid removed, left adrenal removed, right splanchnic cut	Splanchnic stimulation	2:41	2.21		0.83	653	176	36.6	
		2:57	2.24		0.75	632	180	36.5	3:10–3:21 Remaining adrenal removed
		3:52	2.07		0.84	707	194	36.6	
11:40 Preparation complete		4:04	2.07		0.87	776	182	36.5	
	4:20–4:37	4:21	1.88		0.67	509	198	36.4	
	Splanchnic stimulation	4:26	2.24	+8.0	0.80	677	200	36.5	
		4:47	2.71		0.80	880	199	37.0	

30		1:22	1.90		0.77	2068	172	37.0	
5–25–23		1:36	1.82		0.65	1084	222	37.0	
3.4 kg.	1:55–2:07	2:00	2.17	+19.0	0.96	1850	124	37.0	See figure 1
Thyroid removed, left adrenal removed, right denervated.	Splanchnic stimulation	2:22	1.45		0.85	1639	156	37.0	
		2:37	2.07		0.70	1621	138	37.2	2:48–2:55 Remaining adrenal removed.
		2:57	2.00		0.79	1462	132	37.1	
Nerves to the liver cut 11:35 Preparation complete		3:25	1.88		0.83	1306	152	37.0	3:52 The low R.Q.'s shown in this and the two succeeding periods may be the result of the marked decrease in ventilation that occurred at this time. This would not greatly alter the caloric determinations, since these are based on oxygen consumption and calculated in this instance on a minimum R.Q. of 0.72
		3:43	1.82		0.73	1208		37.0	
	3:52–4:08	3:52	1.32	−27.5	0.58	621	130	37.0	
	Splanchnic stimulation	3:58	1.79		0.70	796	146	37.0	
		4:15	1.80		0.64	695	154	37.0	
31		3:16	1.25		0.67	970	127	37.0	Experiment carried out as a special control to test effect of splanchnic stimulation with adrenals removed and nerves to liver cut
5–31–23		4:37	1.59		0.86	2030	156	37.2	
3.52 kg.		4:50	1.83		0.71	1929	136	37.4	
Thyroid removed; nerves to liver cut; both adrenals removed and right splanchnic prepared for stimulation 2:58 Preparation complete	5:03–5:19	5:03	1.33	−27.5	0.68	1113	132	37.1	
	Splanchnic stimulation	5:09	1.45		0.86	1223	168	37.0	
		5:24	1.58		0.79	1750	142	37.0	
		5:35	1.60		0.82	1953	150	37.0	

TABLE 1—*Concluded*

EXPERIMENT NUMBER, DATE, WEIGHT, PRELIMINARY OPERATION	STIMULATION	TIME	CALORIES PER KILO	VARIATION	R.Q.	VENTILATION	PULSE	TEMPERATURE	REMARKS
				per cent		*cc./min.*		*°C.*	
32		11:05	1.98		0.76	1786	124	37.9	10:40 Hepatic nerves prepared for stimulation
6–5–23		11:57	1.73		0.72	869	132	38.1	
3.6 kg.		12:10	2.01		0.77	1653	140	38.1	Slight increase in metabolism on stimulating hepatic nerves. Note also that the rise caused by splanchnic stimulation in absence of the adrenals did not occur after the hepatic nerves had been cut
Thyroid removed, both adrenals removed and right splanchnic prepared for stimulation	12:40–12:57	12:40	2.12	+5.4	0.74	1796	148	38.0	
	Stimulation	12:47	1.86		0.76	1525	140	38.0	
	of hepatic	1:00	1.66		0.84	1191	148	38.0	
	nerves	1:22	1.68		0.75	894		38.0	
9:21 Preparation complete	1:35–1:49	1:40	1.85	+10.0	0.82	1163	131	38.0	
	Splanchnic stimulation	1:56	1.84		0.66	840	151	38.0	
	2:56–3:11	2:40	1.71		0.74	944	158	38.2	2:13 Hepatic nerves cut
	Splanchnic stimulation	3:01	1.72	±	0.80	981	160	38.2	

RESULTS. *Presentation of data.* To make graphic the procedures and results, certain experiments having features which we wish to stress have been selected and are presented in figures 1 to 5. We have tried to make these figures as comprehensive as possible in themselves, showing blood-pressure changes, pulse rate, body temperature and time intervals; but for the sake of comparison they are also presented in table 1. Table 1 includes all the complete experiments and really consists of abbreviated protocols. No experiment has been included in which the animal was in poor condition from low blood pressure or other cause.

The more important results in all experiments are summarized in table 2. These are given as averages; and since there are rather wide variations in some of the results summarized, this method is open to criticism. It is useful, nevertheless, in condensing the large volume of data under consideration; and no one need misjudge the averages, for the figures from which they have been calculated are given in detail in the figures and in table 1.

TABLE 2

Number of experiments showing increased metabolism during adrenal stimulation	24
Average percentage increase in metabolism	20.2
Number of experiments showing no increase in metabolism during adrenal stimulation (see text)	5
Average percentage drop in metabolism following removal of adrenals	7
Average body temperature (°C.)	37.3

General considerations. It became apparent early in the work that stimulation of the adrenals was capable of increasing metabolism to a considerable degree. In figures 1 to 4 it will be noted that the metabolism rose during stimulation 19, 35.3 (av.) 50 and 24 per cent, respectively.

It is obvious that when nerves such as the sciatic, brachial or splanchnic are stimulated, other changes in addition to the liberation of adrenin occur in the body economy. Some of these changes are in the heart rate, blood pressure and respiration. Since increased function cannot occur without an increase in metabolic activity, a query is raised as to the effect of the above mentioned factors on the total metabolism. The evidence at hand indicates that their influence is small.

The changes in heart rate and blood pressure when stimulation is applied to the sciatic nerve are of about the same magnitude, whether or not the adrenals are present. It will be noted in figure 4 that the changes in heart rate and blood pressure were not proportionately reflected in the metabolic determinations. Additional evidence as to the small contribution made by the increased work of the heart is furnished by calculations based on formulae given by Evans and Matsouka (7) showing that the increased work of the heart incident to the faster rate could account for only a negligible fraction of the increased metabolism that was obtained.

The respiratory changes that occurred are not shown on the charts. In general, the movements are slow and shallow during an initial period of stimulation; even in some instances amounting to a short interval of apnea. Accompanying this period of interference with the respiratory function there is a lowering of the metabolic level. Following this phase of lowered respiration there is one in which both rate and depth are increased. Regardless of whether or not the first phase occurred, stimulation with the adrenals present almost invariably resulted in an increased ventilation; consequently, in all later experiments here described it was the custom to collect the sample for metabolic determination after stimulation had been in progress five or ten minutes, thus eliminating the period of depressed respiration. Tompkins, Sturgis and Wearn (8) in their experiments noted a very striking increase in respiration following the injection of adrenalin, and they conclude, after a discussion of the controls, that this is the result of the increased metabolism and that the work of the respiratory muscles contributes very little to the increased heat production. This is substantially our interpretation, and is in accordance with the well-known fact that increased ventilation is one of the most constant accompaniments of an increased metabolic rate.

The reason for the preliminary depression of respiration is not clear. In these experiments it was sometimes present when stimulation was applied to the splanchnic after the removal of the adrenals, although under these conditions the phenomenon was not so constant nor of such striking character. Roberts (9) in some recent work on the effects of large doses of adrenalin on the respiratory center reports diminution and temporary arrest of the respiratory movements—facts which are in accordance with the reports of previous observers. He attributes these results to sudden anemia of the respiratory center due to vascular constriction. Nice, Rock and Cartwright (10), working with doses of adrenalin within "physiological" range, find that amounts strong enough to produce a rise in blood pressure result in an increase in the depth of respiration, which may or may not be preceded by a period of shallow respiration; they confirm the findings of others as regards the larger dosage.

Respiratory quotient. Accompanying the increased metabolism occasioned by stimulation of the adrenals, there was an increase in the respiratory quotient of 12.5 per cent. That adrenalin should cause an increase in the respiratory quotient is in accordance with the observations of Boothby (1) and of Tompkins, Sturgis and Wearn (8). Nevertheless, in the experiments here reported it did not seem wise to attach too much importance to this increase, because of the relatively short periods over which the samples were collected; a considerable part of the increased CO_2 output recorded might be due to a "washing out" of CO_2 by the increased ventilation, rather than to an actual change in the proportion of the different foodstuffs burned.

Conditions favoring maximum metabolic rise. Stimulation of the adrenal glands in 24 cases, as shown in table 2, resulted in an increase in metabolism averaging 20.2 per cent. As will be apparent on consulting table 1, this rise varied considerably in the different experiments. As shown by the denervated heart preparation, a liberation of adrenin uniformly resulted from stimulation, so that it was evident that certain other conditions were the varying factors; and much time was devoted to a study of the circumstances under which a maximum rise occurred. It may be well to summarize the findings here, although most of the points have already been discussed.

First: there should be a preliminary denervation of the adrenals. This was not carried out in the 5 cases that failed to show a rise. Second: ample time should be allowed after completion of operative procedures before taking the preliminary metabolic readings. We found in several instances that when stimulation was carried out shortly after the completion of operative procedures, no rise resulted; whereas when the stimulation was repeated after an interval of waiting, an increase in metabolism occurred. Third: the expired air should not be collected until stimulation has been in progress for at least five minutes; otherwise the preliminary period of depressed respiration and metabolism will be recorded (fig. 4).

The more rapid metabolism occasioned by the so-called "specific dynamic action" of certain of the amino acids and of glucose, is well known; and experiments 13, 14, and 15 (table 1) were conducted in which solutions of these substances were injected, either during the stimulation period or a short time before. These solutions were administered with the idea that their presence in the circulating blood in considerable concentration might strikingly increase the effects of the adrenin; but, as may be noted, they had little or no effect.

Mode of action of adrenin. As regards the mechanism whereby adrenin increases the metabolism of the body, sufficient evidence is not afforded by these experiments for definite conclusions to be drawn; but certain factors have been observed. In 10 experiments the thyroid was removed as a preliminary step; so it is certain that adrenin does not exert its effect by stimulating that organ to increased activity. In one case the rise occurred although the liver had been removed after ligating the lobes just above the entrance of the veins into the inferior vena cava, which suggests that the liver is not essential for the response. On the whole we are of the opinion that adrenin stimulates all the body cells to increased metabolic activity.

CONCLUSIONS

1. Urethane acts as a stimulant to the adrenal glands, probably by its effect on the central nervous system.

2. Removal of the adrenals under urethane anesthesia results in a fall of metabolism.

3. Direct splanchnic or reflex stimulation of adrenal secretion results in a prompt increase of metabolism averaging in these experiments 20.2 per cent.

4. According to our evidence, adrenin does not require the coöperation of the thyroid in producing an increased metabolism.

We wish to take this opportunity of thanking Dr. W. B. Cannon for his interest and active aid in carrying out this research.

BIBLIOGRAPHY

(1) BOOTHBY AND SANDIFORD: This Journal, 1923, lxvi, 93.
(2) CANNON AND RAPPORT: Ibid., 1921, lviii, 2, 308.
(3) KROGH: Internat. Zeitschr. f. physik.-chem. Biol., 1914, i, 491.
(4) AUB, BRIGHT AND URIDIL: This Journal, 1922, lxi, 300.
(5) AUB, BRIGHT AND FORMAN: Ibid., 1922, lxi, 349.
(6) CANNON AND CARRASCO-FORMIGUERA: Ibid., 1922, lxi, 2, 215.
(7) EVANS AND MATSOUKA: Journ. Physiol., 1915, xlix, 378.
(8) TOMPKINS, STURGIS AND WEARN: Arch. Int. Med., 1919, xxiv, 269.
(9) ROBERTS: Journ. Physiol., 1921, lv, 346.
(10) NICE, ROCK AND CARTWRIGHT: This Journal, 1914, xxxiv, 326.

THE COLLOID CHEMISTRY OF PROTOPLASM

III. The Viscosity of Protoplasm at Various Temperatures

L. V. HEILBRUNN

From the Department of Zoölogy, University of Michigan, Ann Arbor

Received for publication January 14, 1924

Colloids differ very widely in their relation to temperature. Gelatin in water changes from a gel with high viscosity to a sol with relatively low viscosity, as the temperature is raised. In other cases there is a coagulation effect produced by a rise in temperature.

In living protoplasm it was found by F. and G. Weber (1) for Phaseolus (bean) cells, that a rise in temperature caused a slight decrease in viscosity. Heilbrunn (2) showed that cold also produced a lowering of viscosity in sea-urchin eggs, and this result was confirmed for slime molds by Heilbronn (3). On the other hand, Weber and Hohenegger (4) have confirmed some earlier results of Weber's and have shown that low temperatures may produce an increase in viscosity.

Although there are isolated measurements of protoplasmic viscosity, no one has systematically attempted to trace the variation of protoplasmic viscosity over the entire range of temperatures through which life is possible. It was thought that such a study would prove valuable.

The material used was protoplasm of the Cumingia egg. (Cumingia is a small clam found at Woods Hole.) This protoplasm is particularly suitable for viscosity determinations by the centrifuge method. The principles governing the use of the centrifuge method have been discussed in previous publications (5) and need not be considered again. The essential feature of the method as it is ordinarily used, is that granules are moved through the protoplasm by the action of a given centrifugal force, and the time necessary to move the granules through a given distance in the egg is recorded. A hand centrifuge, such as that manufactured by Bausch and Lomb, is convenient and it may be turned at various speeds. In earlier work the high speed handle was usually turned once per second, but this speed is too high for a comparison of slight differences in the viscosity of Cumingia egg protoplasm. For these tests, therefore, a speed only one-fourth as great was used, and the high speed handle was turned once every four seconds. This gave a force 311 times gravity. Such a slow rate of centrifuging gave greater precision, but it had the disadvantage that a much

longer time was required for the tests. This becomes of importance at temperatures relatively far from the room temperature, especially in those cases in which the viscosity is high. Thus the determinations which were made at 0 degree and at 1 degree as well as that made at 31 degrees, are not very reliable. They are to be considered more as minima than as actual values, although check tests at higher speeds showed that the tests at the lower temperatures are not very far wrong.

The results of the viscosity determinations are given in the form of a curve (fig. 1). This curve is the first one that has ever been made to show the variations of protoplasmic viscosity at different temperatures. No claim is made for any great accuracy. It is believed that the main facts shown by the curve are correct, but the individual measurements are not very accurate. It is not yet possible to measure protoplasmic viscosity with anything like the accuracy possible in the measurement of the viscosity of ordinary liquids. However the changes in the viscosity of protoplasm at different temperatures are so striking, that even a comparatively inaccurate method shows them beyond any possible question.

In the curve the abscissae represent temperatures. For temperatures above that of the room, the temperature was controlled by a de Khotinsky constant temperature apparatus. At lower temperatures the eggs were placed in a tube surrounded by ice water.

The ordinates represent viscosity as measured by the number of seconds of centrifugal turning necessary to move the granules in the egg to such an extent that the appearance of zones was produced. Each point on the curve was the result of a series of measurements. Thus at 23 degrees it was shown that 17 turns of the centrifuge (for 68 seconds) did not produce zones in the egg, whereas 18 turns (72 seconds) did produce zones. The viscosity was therefore given at 72, for the limits of accuracy of the method did not make it advisable to differentiate any closer than this.

Study of the curve shows that there is low viscosity both above and below a maximum at about 15 degrees. As the temperature rises above 15 degrees or falls below it, the protoplasm becomes more and more fluid until suddenly it undergoes a sharp increase in viscosity. This is essentially the same result that was obtained by Heilbronn in his study of the protoplasm of slime moulds.

The curve of protoplasmic viscosity in the Cumingia egg does not of course give an accurate representation of the protoplasmic viscosity of all types of protoplasm. In the Arbacia egg the protoplasm undergoes a sharp increase in viscosity at a lower temperature than does the Cumingia egg. This is to be associated with the fact that the curve which has been plotted for the Cumingia egg does not apply accurately to all types of protoplasm; it is nevertheless apparent that the general relation of protoplasmic viscosity to temperature is much the same in widely different sorts of protoplasm.

The fact that a sharp increase of viscosity may occur a degree or two above the freezing point may offer an explanation of various puzzling phenomena. The plant physiologist, Sachs, was at a loss to explain the apparent freezing of tropical plants at temperatures above the freezing point (6). He was inclined to doubt the truth of this old observation. The facts of this paper indicate that there may be coagulative change at temperatures

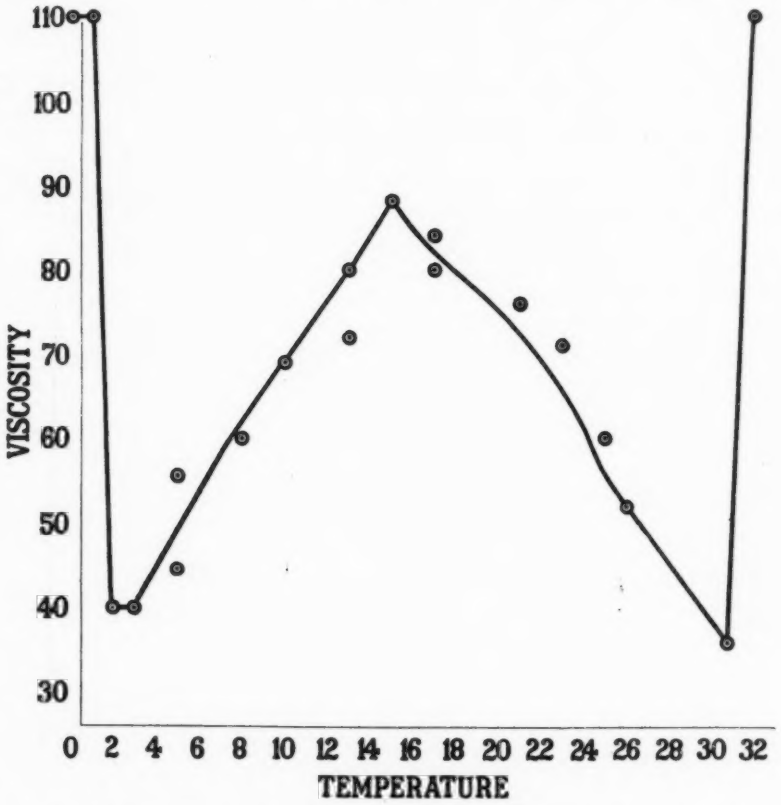


Fig. 1. The viscosity of Cumingia egg protoplasm at various temperatures

above the freezing point, and they thus furnish a reasonable explanation of the data cited by Sachs. Perhaps also the contraction of muscle produced by low temperature, and the occasional cases in which cold causes artificial parthenogenesis may be interpreted as due to a coagulative effect of the cold. The fact that Plough's (7) curve of the relation of crossing-over in Drosophila to temperature is roughly similar to the curve of protoplasmic viscosity may also be significant.

SUMMARY

A curve has been plotted which shows the relative viscosity at various temperatures of the protoplasm of the Cumingia egg.

BIBLIOGRAPHY

(1) WEBER AND WEBER: Ber. d. Deutsch. Botan. Gesell., 1916, xxxiv, 836.
(2) HEILBRUNN: Biol. Bull., 1920, xxxix, 307.
(3) HEILBRONN: Jahrb. f. wiss. Bot., 1922, lxi, 284.
(4) WEBER AND HOHENEGGER: Ber. d. Deutsch. Botan. Gesell., 1923, xli, 198.
(5) HEILBRUNN: Journ. Exper. Zoöl., 1920, xxx, 211; ibid., 1921, xxxiv, 417.
(6) SACHS: Handbuch der Experimental-Physiologie der Pflanzen, Leipzig, 1865. See pp. 57–58.
(7) PLOUGH: Journ. Exper. Zoöl., 1917, xxiv, 147.

cal Society under the direction of the Council of the Society. From three to four volumes each of about six hundred pages are published yearly. The subscription price per volume in the United States and Canada is $5.00; in other countries $5.25.

Manuscripts summitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, 19 W .Chase Street, Baltimore, Md. They should be packed flat, carefully protected from injury and insured.

Each article should conclude with a brief summary of results suited to the needs of reference journals.

References to literature cited should contain: first the name of the author or authors, second the name of the journal or book; third the year; fourth the volume number and fifth the page. These references should be collected on a separate page at the end of the article with corresponding bracketed numbers in the text. Foot notes are to be avoided whenever possible.

All figures for illustration should be submitted in such form as to admit of photographic reproduction without retouching or redrawing. Marginal letters to figures cannot be set in type and should, therefore, be written in India ink and regarded as a part of the figure. When artist's work is required to make figures suitable for reproduction this will be charged to authors. Unless specific instructions are given by authors the printer will be allowed to use his judgment in determining the amount of reduction on all figures. In any case the plates should never exceed the dimensions of the printed matter of the JOURNAL page unless the author is prepared to pay the cost of an insert page.

Tracings on smoked paper reproduce well if the white lines are distinct and separate and the background uniform black. Authors may safely retouch breaks or weak spots in the white lines with white ink and fill in the background with India ink. Curves laid on coördinate paper should be in India ink. Background lines in blue will not reproduce; red, yellow, and green will reproduce black. Plotted curves and drawings yield better results if the originals are large enough to permit of considerable reduction in the plates used for illustration, but authors are warned to make due allowance for this in the breadth of lines and size of lettering.
